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THE EFFECT OF NARCOSIS ON ELECTROTONUS

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Pflüger showed that during the passage of a galvanic current through a nerve the physiological condition of the nerve in the neighborhood of the poles is changed. Currents—"electrotonic currents"—are set up near both poles. In the region around the anode the irritability, as measured by the threshold for stimulation, and the conductivity, as measured by the speed of the nerve impulse, are decreased; in the region around the cathode, provided the current is not too strong, the irritability and the conductivity are increased. If the current is long continued, however, the cathodal hyper-irritability and hyper-conductivity are lessened and may even change to sub-normal values; such sub-normal values appear very quickly or at once if the polarizing current is very strong.

The above effects are those observed in non-narcotized nerve. It should also be of interest to examine what effects are produced by the electric current in nerve that has been narcotized.

In regard to the electrotonic currents, Waller found that these are set up by a polarizing current even in a "completely" narcotized nerve, although not in a dead nerve, and this is in accord with Sosnowsky's statement that medullated rabbit nerves continue to show electrotonic currents for several days after they have lost their ability to conduct an impulse.

The question then arises: How does narcosis affect the changes in irritability and conductivity that are brought about by a polarizing current? And this in turn raises two questions. What is the nature of electrotonic changes in irritability and conductivity in the "partially" narcotized portion of nerve itself, that is, in the region of decrement? And, does a "completely" narcotized stretch of nerve block the spread of the electrotonic change in irritability and conductivity to regions beyond in the same sense at it blocks the spread of the nerve impulse? The answers to these questions are furnished by the following experiments.

Let us first consider electrotonic changes of *conductivity* in a region of decrement.

The sciatic nerve of a frog muscle-nerve preparation was passed through small holes in the sides of a narcotizing chamber (an ebonite chamber, about 1 cm. in diameter) (fig. 1). The holes were plugged carefully with a thick paste of kaolin moistened with Ringer's solution. By this arrangement the portion of the nerve in the chamber was separated from the rest of the nerve, so that it alone might be exposed to the action of a narcotic, e.g., ether fumes. *A* and *B*, both proximal to the chamber, were non-polarizable boot electrodes of the polarizing circuit; *A*, the more proximal electrode, being the anode, and *B*, the more distal electrode, the cathode. *B* was placed in close enough proximity to the narcotizing chamber for catelectrotonic changes to spread from it over the stretch of nerve contained within. At *I*, between the distal electrode and the narcotizing chamber, were platinum electrodes connected with an inductorium. The whole was placed in a moist chamber. The source of the polarizing current was two ordinary dry cells (about two volts). This strength of current caused increased irritability at the cathode, as measured by the minimal strength of induction shock necessary to cause muscular contraction. The inductorium was now set so that the stimulus was above the threshold. In the following table, which is a protocol of one of the experiments, R signifies muscular contraction; r, no muscular contraction; the first column, I, shows whether or not the induction shock caused muscu-

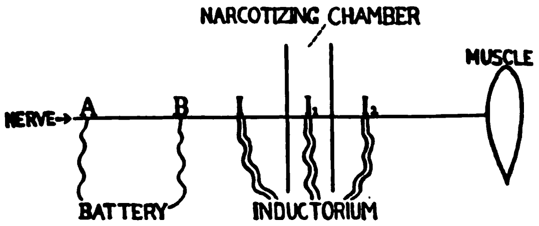

Fig. 1

lar contraction when applied in the absence of the polarizing current; column M shows whether or not muscular contraction occurred at the make of the polarizing current; IC shows the result from the induction shock applied while the polarizing current was passing through the nerve; B shows the result at the break of the polarizing current. The stimuli were applied in cycles in this order and sufficient time was allowed between stimuli for recovery in the muscle. The numbers in the table refer to the successive cycles of stimuli. At the point marked "narcosis started," air bubbled through ether and water was passed through the narcotizing chamber; at the first point marked "partial narcosis," the ether in the chamber was replaced by air bubbled through water.

TABLE 1

Narcosis applied to the region subject to catelectrotonus

	I	M	IC	B
1. Without narcosis	R	R	R	R
2. Narcosis started	R	R	R	R
3. Complete narcosis	r	r	r	r
4. "Partial" narcosis	R	R	r	r
5. "Partial" narcosis	R	R	r	r
6. "Partial" narcosis	R	R	r	r
7. "Partial" narcosis	R	R	r	r
8. "Partial" narcosis	R	R	r	r
9. "Partial" narcosis	R	R	r	r
10. "Partial" narcosis	R	R	r	r
11. "Partial" narcosis	R	R	r	r
12. "Partial" narcosis	R	R	r	r
13. "Partial" narcosis	R	R	r	r
14. "Partial" narcosis	R	R	r	r
15. "Partial" narcosis	R	R	r	r
16. "Partial" narcosis	R	R	r	r
17. Recovery complete	R	R	R	R
18. Recovery complete	R	R	R	R

1, in table 1, shows that an induction shock (above the threshold) called out muscular response, both without and with the polarizing current acting; it shows also that muscular contraction occurred both at the make and at the break of the polarizing current. This is the condition normally found in the nerve, in the case of a moderate polarizing current, and may be called stage N. Ether fumes were then started through the narcotizing chamber. At 3, all impulses failed to get through the region of decrement. This may be called stage C, "complete" narcosis. The ether was now displaced by air saturated with water vapor. The portion of the nerve in the chamber now began to regain its conductivity. In this stage of "partial" narcosis (stage P), from 4 to 16 inclusive, induction shocks of the same strength as those used in 1, caused muscular contraction if applied in the absence of the polarizing current, but failed to do so if applied while the polarizing current was passing through the nerve. At the same time muscular response occurred at the make of the polarizing current but not at the break. After still further recovery of conductivity (17 and 18), the polarizing current failed to prevent muscular response to an induction shock; at this time muscular contraction occurred at the break of the polarizing current as well as at the make. Thus the nerve had reëntered stage N. Experiments have shown that stage P, partial narcosis, may be gotten not only when the nerve is coming out of narcosis, but also when it is going into narcosis.

The chief point brought out by this experiment is that—whereas in the non-narcotized region of the nerve catelectrotonus involves an increase of conductivity for the impulse—*in the region of the nerve under partial narcosis catelectrotonus involves a decrease of conductivity; the conductivity change of catelectrotonus thus becomes reversed in sign under narcosis;* for, during the stage called "partial narcosis," impulses are not blocked in the region of decrement so long as the polarizing current is off—contraction regularly following stimulation, but impulses are blocked at the region of decrement during the passage of the polarizing current, and no muscular contraction can be obtained. The reason why contraction occurs at the make but not at the break will be considered later.

The effect of anelectrotonus on conductivity, in a region of decrement, is shown by an experiment similar to that above, but with A the cathode and B the anode (see fig. 1). The results are shown in table 2.

As in the case of catelectrotonus, three stages are seen as the depth of narcosis increases towards "completeness," they are also seen during recovery: Stage N: Before the exposure of the nerve in the chamber to ether fumes, muscular contraction followed an induction shock, both when the shock was applied in the absence and when it was applied in the presence of the polarizing current (1 in table 2). Stage P: After a certain degree of narcosis was reached (3, 4, 5), the induction shock called out a muscular response if applied in the absence of a polarizing current but failed to do so if applied during the passage of the polarizing current. At the same time response occurred at the break but not at the make of the polarizing current; this is the reverse of what is gotten in the corresponding stage when the cathode is nearer the chamber. Stage C: All impulses fail to get through. During recovery the opposite sequence of stages was observed.

From this experiment it is evident that anelectrotonus, like catelectrotonus, decreases conductivity in a region of decrement. As anelectrotonus normally decreases conductivity, however, it is evident that the anelectrotonic conductivity change, unlike that of catelectrotonus, is not reversed in sign under narcosis; it summates with narcosis.

Nos. 2 and 9 (table 2) bring out a rather unexpected point. In these cycles of stimuli, muscular contraction did not occur at the make of the polarizing current although it did occur when a stimulus was applied during the passage of the polarizing current, and it did occur at the break. Hence the decrease of conductivity in the region of decrement is greater immediately after closing the polarizing current than it is later on during the passage of the current or immediately after the break.

TABLE 2

Narcosis in region subject to anelectrotonus

	I	M	IC	B
1. Without narcosis	R	R	R	R
2. Narcosis started	R	r	R	R
3. "Partial" narcosis	R	r	r	R
4. "Partial" narcosis	R	r	r	R
5. "Partial" narcosis	R	r	r	R
6. Narcosis "complete"	r	r	r	r
7. "Partial" narcosis	R	r	r	R
8. "Partial" narcosis	R	r	r	R
9. "Partial" narcosis	R	r	R	R
10. Recovery "Complete"	R	R	R	R

We may now consider why the make and the break of the polarizing current do not have the same effects. For this purpose tables 1 and 2 may be put in the form of Pflüger's law of stimulation by moderate ascending and descending currents. This is shown in table 3. It will be seen here that narcosis causes a modification of Pflüger's law, of such nature that, when there is a region of decrement, the electrotonic effect of a moderate current is similar to the ordinary effect of a very strong current.

In the case of the descending current, the make stimulus, arising at the cathode *B*, arrives at the region of decrement before catelectrotonic decrease of conductivity appears in that region, so that the impulse gets through. A longer interval of time than that taken by the nerve impulse in traveling from *B* through the region of decrement must therefore elapse before the full catelectrotonic decrease of conductivity, also originating from *B*, appears in the region of decrement. The break impulse, arising from the anode *A*, arrives at the region of decrement before the catelectrotonic decrease of conductivity disappears, so that the impulse is put out.

In the case of the ascending current, on the other hand, by the time the make stimulus, arising at the cathode *A*, arrives at the region of decrement, anelectrotonic decrease of conductivity, originating from *B*, has already appeared in that region, so that the impulse is blocked. By the time the break impulse, which arises at the anode *B*, arrives at the region of decrement, anelectrotonic decrease of conductivity has disappeared, and the impulse gets through.

TABLE 3

Pflüger's law for moderate currents, as affected by narcosis

	ASCENDING CURRENT (FROM TABLE 2)		DESCENDING CURRENT (FROM TABLE 1)	
	Make	Break	Make	Break
Without a region of decrement	Response	Response	Response	Response
With region of decrement	No response	Response	Response	No response

An idea of the time required for the appearance of anelectrotonus in a distant extra-polar region may be gotten in the following manner. In table 2 it was seen that the make impulse, starting at *A* (fig. 1), failed to get through when the stretch of nerve in the chamber was partially narcotized. Now, if the time interval which elapses before the appearance of anelectrotonus is greater than that required for a nerve impulse to travel from *B* to the region of decrement, then it would be possible to get muscular contraction on the make of the polarizing current, as well as on the break, by bringing *A* sufficiently near *B*. In fact there would be a position for *A* such that the time required for the impulse to travel from that point *A*, through the region of decrement, is equal to the time required for anelectrotonus from *B* to appear in the region of decrement.

In the following experiment *B* was 2 cm. from the narcotizing chamber. After a region of decrement was established by narcosis, muscular contraction occurred only at the break of the polarizing current, until *A* was brought within 0.5 cm. of *B*, and thus within 2.5 cm. of the narcotizing chamber; now muscular contraction occurred both at the make and at the break of the polarizing current. On moving *A* back further from *B*, a response occurred only at the break. (Five dry cells were used in the polarizing circuit.) Approximately the same results were obtained in six other similar experiments. This shows that the time required for anelectrotonus to appear 2 cm. from the anode, in amounts sufficient to block an impulse in a region of decrement 0.5 cm. long, is equal to the time required for the nerve impulse to travel a distance slightly over 2.5 cm., plus the refractory period of the nerve. In estimating this time it should be remembered that the impulse has to travel through an anelectrotonic stretch of nerve, in which the speed of conduction is considerably reduced.

The time required for the disappearance of anelectrotonus from a region of decrement is much shorter. This is shown in experiments with ascending currents, in which the anode was as near the narcotizing chamber as 0.5 cm.; during the passage of the polarizing current the anelectrotonic effect was sufficient to block the impulses in the region of decrement, yet at the break a response was obtained—the anelectrotonus had disappeared before the impulse had traveled 0.5 cm. It will be observed that this is in marked contrast to the result obtained with catelectrotonus,—in that case, the anode being at A, the impulse started at the break had to travel a considerable distance (4 cm.) before it reached the narcotized region; nevertheless that region was still so affected by the catelectrotonus that the impulse failed to get through.

Further experiments showed that the after-effect of anelectrotonus in a region of decrement is an increase in conductivity. After narcosis has progressed to such a point that impulses from the proximal portion of the nerve are blocked in the region of decrement even when the polarizing current is off, nevertheless they will still go through if they arrive at the region of decrement soon after the breaking of the polarizing current. In an experiment illustrating this effect the anode, which was, as before, in position *B*, was placed at about 1 cm. from the narcotizing chamber; the cathode was placed 4 cm. from the anode. Ether was passed through the chamber until no response was obtained from induction shocks applied between *A* and *B*. The inductorium electrodes were then removed and the galvanic current was closed; no muscular response occurred on the make. On the break of the polarizing current, however, muscular contraction occurred. Soon afterwards "complete" narcosis made its appearance. Tests made during recovery in the narcotized stretch gave the following: first, contraction occurred on the break of the galvanic current, but not from induction shocks, nor on the make of the current. Then there was a time during which muscular response followed induction shock, as well as the break of the polarizing current; but no muscular response occurred at the make. Finally, muscular contraction occurred at the break of the polarizing current, from the induction shock, and at the make. This shows conclusively that a portion of the nerve so deeply narcotized as to block all impulses may be made conductive as an after-effect of anelectrotonus (i. e., at the break of the current). The change from decreased to increased conductivity must take place very quickly after breaking the current.

The electrotonic changes in *irritability* during narcosis may next be considered. Those which occur in the case of catelectrotonus will be dealt with first.

Electrodes connected with an inductorium were placed *inside* the narcotizing chamber (at point I_1, fig. 1), in contact with the nerve. The anode of the polarizing current was at A, the cathode at B, so that the region to be etherized might be subjected to catelectrotonus. At first the irritability of this portion of the nerve was tested without a narcotic. It was found that with the polarizing current on, irritability in this region was increased as measured by the strength of induction shock necessary to cause muscular response. The inductorium was then set at a strength above the threshold necessary for stimulation while the polarizing current was off, and air bubbled through ether was now passed through the narcotizing chamber. As narcosis in the region of decrement gradually progressed, stimuli from the inductorium were sent in alternately without and with the polarizing current on. A typical protocol was as follows:

Results from stimulation within a partially narcotized region, subject to catelectrotonus, narcosis progressively increasing

	POLARIZING CURRENT OFF	POLARIZING CURRENT ON
1	Response	Response
2	Response	Response
3	Response	No response
4	Response	No response
Stronger induction shock		
5	Response	Response
6	Response	Response

In 3 and 4 an induction shock applied during the passage of the polarizing current did not cause muscular contraction, although in the absence of the polarizing current an induction shock of the same strength did produce a muscular response. This might be due to electrotonic decrease of either irritability or conductivity; that is, the stimulus may have become subminimal and failed to start an impulse, or the impulse, once started, may have been blocked. Nos. 5 and 6 show that the impulses were not blocked, for it required merely a stronger stimulus in order to obtain a muscular response: irritability, therefore, had become decreased under catelectrotonus. *Thus, under narcosis, not only is the conductivity change of catelectrotonus reversed in sign, but also the irritability change.*

Similar experiments showed that anelectrotonus too decreases irritability in the region of decrement. Here again we see that the effects on irritability run parallel with those on conductivity.

Experiments were also undertaken to determine whether electrotonus can "spread" from its pole of origin, through a narcotized region, out into a normal region beyond. For this purpose the inductorium electrodes were placed at I_2 (fig. 1), on the other (distal) side of the narcotizing chamber from the polarizing electrodes A and B. Table 4 (a) shows the effect of anelectrotonus from B on irritability at the point I_2; table 4 (b) shows the effect of catelectrotonus from B on irritability at the same point. In both cases ether was the narcotic used and the portion in the chamber was "completely" narcotized, no muscular contraction resulting when the polarizing current was opened or closed.

It is quite evident here that both anelectrotonus and catelectrotonus are "transmitted" unchanged in nature through the region under narcosis, even though in this region itself they may be reversed in sign and although impulses in this region are blocked.

Experiments using as the narcotic chloral hydrate, magnesium sulphate, ethyl alcohol and zinc sulphate (saturated solution), all gave similar results. In many of these experiments the region in the narcotizing chamber had been so long under narcosis, and had been subjected to such high concentrations of the narcotic, that it must almost certainly have been "dead;" later, it failed to recover from the effects of the narcotic and could not be made to conduct an impulse. Nevertheless, electrotonus was transmitted through these regions.

TABLE 4

The spread of electrotonus through a narcotized region

(a) Anelectrotonus: Anode at *B* (fig. 1)

	POLARIZING CURRENT OFF; THRESHOLD STIMULUS FROM INDUCTORIUM	POLARIZING CURRENT ON; STIMULUS OF SAME STRENGTH AS IN FIRST COLUMN
1	Response	No response
2	Response	No response
3	Response	No response
4	Response	No response
	etc.	

(b) Catelectrotonus: Cathode at *B*

	POLARIZING CURRENT OFF; STIMULUS FROM INDUCTORIUM JUST BELOW THRESHOLD	POLARIZING CURRENT ON; STIMULUS OF SAME STRENGTH AS IN FIRST COLUMN
1	No response	Response
2	No response	Response
3	No response	Response
4	No response	Response
	etc.	

It should be noted, however, that in one experiment, after soaking the portion of the nerve in the narcotizing chamber in zinc sulphate for two hours, electrotonic changes in irritability failed to spread through to the point of stimulation, although they did so for quite a time (thirty minutes) after conductivity had been abolished. Moreover, it was found that if the nerve is cut, instead of having a portion narcotized, the ends being placed close together, electrotonic changes in irritability started in the free piece of nerve do not get into the portion of the nerve attached to the muscle. Accordingly we must conclude that the processes whereby electrotonus spreads from the polarizing electrode, although not dependent upon properties peculiar to the functioning or the living nerve, nevertheless require some structure or substances other than the grosser physical and chemical characteristics of the nerve.

The following diagram sums up the electrotonic changes occurring *during*[1] the passage of the polarizing current in the three portions of the nerve: *a*, In the non-narcotized proximal portion around the distal polarizing electrode; *b*, in the region of decrement; *c*, in the non-narcotized portion distal to the narcotizing chamber.

WITH ANELECTROTONUS			WITH CATELECTROTONUS		
Region near electrode	Region of decrement	Distal region	Region near electrode	Region of decrement	Distal region
Irritability decreased	Irritability decreased	Irritability decreased	Irritability increased	Irritability decreased	Irritability increased
Conductivity decreased	Conductivity decreased	Conductivity decreased	Conductivity increased	Conductivity decreased	Conductivity increased

SUMMARY

1. Whereas, in a non-narcotized portion of the nerve, a moderate polarizing current causes an increase of irritability near the cathode, in a narcotized portion of the same nerve this current causes a decrease in irritability. The effect of catelectrotonus on irritability therefore is reversed during narcosis.

2. A similar reversal of effect occurs in the case of conductivity. Catelectrotonus decreases conductivity in a partially narcotized region.

3. Anelectrotonus causes a decrease in irritability in a partially narcotized portion of the nerve, as in the non-narcotized nerve. Thus there is no reversal of effect as in the case of catelectrotonus.

4. Anelectrotonus similarly decreases the conductivity in a partially narcotized region, as in a non-narcotized region. This decrease

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[1] The effects immediately after the make or break of the polarizing current are not shown here.

of conductivity is especially marked immediately after closing the polarizing current.

5. The time interval which elapses before anelectrotonic decrease in conductivity appears in a distant "partially" narcotized part of the nerve, to such an extent that the nerve impulse is put out, is longer than the time required for a nerve impulse to travel the same distance; thus an approximation of the speed of anelectrotonus (in the above sense) may be arrived at.

6. Concerning the interval which elapses before catelectrotonus causes sufficient decrease in conductivity in a distant narcotized portion to block an impulse, all that can be said is that it is longer than the time required for the impulse to travel the same distance.

7. A block for nerve impulses, set up by narcotizing a portion of the nerve, may be momentarily removed by the after-effect of anelectrotonus (effect produced at the anode immediately after turning off the polarizing current). Hence the after-effect of anelectrotonus in a narcotized portion of nerve is an increase of conductivity; this increase appears very quickly after the breaking of the polarizing current.

8. The after-effect of catelectrotonus in the narcotized portion is a decrease in conductivity.

9. Electrotonic changes are transmitted from a polarizing electrode through an intermediate narcotized region to the non-narcotized stretch beyond. This occurs when the narcosis is so deep that all impulses are blocked and even when the intermediate region of the nerve has been killed by narcosis. The electrotonic effects thus transmitted are of the same sign in the region beyond as they would be in the absence of a block, although in the intermediate region they may be changed in sign, as described above. If, instead of narcotizing, the intermediate portion of the nerve is cut and the ends are closely applied to each other, electrotonic changes in irritability do not spread to the neighboring piece. Prolonged exposure to concentrated zinc sulphate also blocks the spread of electrotonic effects.

For many suggestions and much valuable advice I wish to express my sincere thanks to Dr. H. J. Muller and to Dr. E. Altenburg.

EFFECT OF FATIGUE UPON THE HEART AND CARDIO-SKELETAL QUOTIENT

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On account of certain physiological properties of the heart muscle, it is generally stated that it can not be fatigued. The so-called refractory period is in large part responsible for the ineffectiveness of repeated stimuli to throw the heart into tetanus or fatigue. Skeletal muscles differ from heart muscle in that they very quickly show results attributable to fatigue, whether they are stimulated directly, or indirectly through their motor nerves. Also, one group of muscles may show the effects of fatigue, even though inactive, if other muscles are working and dumping their fatigue products into the circulation. These fatigue products are said to exert the same effects upon a muscle, whether they are produced in the muscle affected or in some distant muscle. That these products affect tissues other than muscle, e.g., nerve cells, is shown by the well known sensation of weariness that arises as a result of fatigue of muscles. The fact that a working group of muscles may, by its fatigue products carried in the circulation, produce manifestations of fatigue in other groups of muscles not working, suggested the question of the heart muscle also being affected by these fatigue products, and if so, would the effects be the same as those caused in other muscles? With this question in mind it was decided to investigate the effect of skeletal muscle fatigue upon the response of the heart muscle to quantitative electrical stimulation, utilizing a change in irritability of the heart muscle as an index of the effect of fatigue.

In a previous paper (1) was suggested the method of using a variation in a body constant as an index of the effects of physiological or pharmacological agents upon the heart or upon the skeletal muscles. The cardio-skeletal quotient was defined as a body constant which represents the relationship existing between the strength of stimulus necessary to arouse skeletal muscle (gastrocnemius) to contraction, and the stimulus necessary to arouse heart muscle to contraction. In the work referred to above it was found that it takes approximately six times as strong a stimulus to make the frog's ventricle contract as it does to make its gastrocnemius contract. The figure found to express the cardio-skeletal quotient in those experiments is 0.17. In the control series of animals in the present investigation the cardio-skeletal quotient coincided exactly with the above figure.

Since the effects of fatigue on the heart were to be studied, it was desirable that in determining the cardio-skeletal quotient, one gastrocnemius be kept as nearly normal as possible. Following is the method used in this investigation. Frogs were used. First the brain was pithed, then the left leg was ligated close to the body. The skin below the ligature was slit and a circular incision made which cut all the tissues down to the bone. This was done in order to prevent any impulses passing under the ligature and thereby producing changes in the gastrocnemius muscle which was used as a control. The fatiguing process was next performed. This consisted in alternately stimulating the stump of the spinal cord and the muscles through the skin until the muscles failed to respond to stimulation through the cord. The cord was then pithed, and if extreme fatigue was desired, the muscles were stimulated through the skin until the responses were very feeble. In some instances where a more normal degree of fatigue was desired, the left leg was ligated first and after waiting a few minutes until pressure anesthesia of the leg had developed, the circular incision was made to the bone. The animal exhibited no signs of pain during this procedure. The frog was then induced to fatigue itself. This was done by placing the frog on its back, from which position it was rather difficult for it to recover because of the loss of function in one leg. It was found that this process could be carried on only for a short time, when the frog apparently would rather lie on his back and rest than turn over; so light electrical stimulation was then applied to the skin. This would arouse the frog to an effort of trying to escape. When this had been continued for some time and the frog showed signs of marked fatigue, the brain and cord were pithed. The heart was then exposed and prepared in the same manner as described in a previous paper (2). The threshold for the ventricle was determined immediately after introducing the electrodes and every five minutes thereafter for a period of twenty minutes; the threshold was then determined for the gastrocnemius immediately after introducing the electrodes and every five minutes thereafter for a period of twenty minutes. In the experiments in the previous series it was found that the irritability of the ventricle and of the gastrocnemius become fairly constant after the lapse of fifteen minutes, and twenty minutes was the interval at which the cardio-skeletal quotient was estimated. In the present series several observations were made extending over a period of forty-five minutes to an hour.

Since in the present series one gastrocnemius was maintained in a normal condition (i.e., unfatigued), a few considerations should first be stated in regard to changes in the cardio-skeletal quotient under the conditions adopted. It is obvious that, the gastrocnemius remaining constant, any variation in the cardio-skeletal quotient must be the result of changes in irritability of the heart muscle. Hence a *decrease* in the cardio-skeletal quotient (i.e., from 0.17 to 0.12) must indicate a decrease in irritability of the heart muscle. The Z units are reciprocally related to the irritability of the muscle, thus, a *decrease* in irritability requires an *increase* in Z units necessary to arouse contraction. Conversely, it may be stated that if the gastrocnemius remains constant, an *increase* in the cardio-skeletal quotient indicates an increase in the irritability of the heart muscle or a *decrease* in the Z units necessary to arouse contraction. In other words, the plotted curve of the cardio-skeletal quotient will parallel the curve of irritability of the heart provided the gastrocnemius is kept constant. The Martin method of quantitative stimulation was used (3). Since the previous investigation showed that the results obtained by the Z units corresponded with the results obtained by the β units, the latter were not estimated in this series. A number of control experiments equal to the number of fatigue experiments were made.

In table 1 are shown the results obtained in the control animals. In the first two columns are shown the threshold, in Z units, of the ventricle immediately after introduction of the electrodes, also the corresponding cardio-skeletal quotient (C. S. Q.). In the succeeding columns are shown corresponding results at five-minute intervals. Table 2 shows the results of a similar procedure in fatigued animals. It will be noted that the average cardio-skeletal quotient in the control animals is the same (0.17) as was found in the series reported previously. This was estimated after the lapse of twenty minutes, as in the preceding series. The average cardio-skeletal quotient in fatigued animals is 0.22. Evidently the irritability of the heart is increased as a result of fatigue, if the cardio-skeletal quotient is estimated at the end of twenty minutes. The average threshold of the ventricles of the fatigued animals, compared with the average threshold of the ventricles of the control frogs shows an increase of irritability amounting to 54.5 Z units or 24.7 per cent. Expressed in terms of the cardio-skeletal quotient, the irritability has increased from 0.17 to 0.22, a difference of 0.05 or 29.4 per cent. It may be that this is too high a per cent; thus it has already been shown (4) that the cardio-skeletal quotient is reliable in estimating the probable Z units of the heart, provided a series is used, thereby obtaining an average. Since the cardio-skeletal quotient, 0.17, in this series of control animals, also in the previous investigation, represented a normal condition, it might well be inferred that it represents the cardio-skeletal quotient in these animals before they were fatigued. On this basis the Z units of the ventricles in the fatigued frogs were estimated from the gastrocnemius Z units, using the cardio-skeletal quotient, 0.17. In these fatigued animals the gastrocnemius was kept normal, i.e., did not take part in the fatigue process. The results of the estimations show the average

TABLE 1

Showing the threshold stimulus of the ventricle in non-fatigued frogs immediately after introduction of the electrodes and at five-minute intervals thereafter for twenty minutes: the corresponding cardio-skeletal quotients (C. S. Q.) are also shown

EXPERIMENT NUMBER	0 MINUTES		5 MINUTES		10 MINUTES		15 MINUTES		20 MINUTES	
	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.
5			161.0	0.27	212.5	0.26	238.0	0.26	255.9	0.25
9	87.5	0.13	156.6	0.08	171.5	0.07	188.0	0.07		
10			287.9	0.11	142.1	0.24	166.5	0.21	176.5	0.20
24	134.5	0.07	101.2	0.23	138.2	0.19	156.6	0.17	161.0	0.17
26	287.9	0.05	109.2	0.22	176.5	0.15	212.5	0.14	220.5	0.15
27	355.5	0.10	188.0	0.13	138.2	0.16	151.8	0.23	151.8	0.23
28	119.1	0.14	246.5	0.09	255.9	0.08	255.9	0.09	265.9	0.09
29	166.5	0.13	123.0	0.22	147.1	0.19	166.5	0.21	181.8	0.22
30	436.5	0.06	188.0	0.14	212.5	0.15	220.5	0.16	203.8	0.19
31	151.8	0.17	138.2	0.18	195.5	0.13	238.0	0.11	299.2	0.09
32	238.0	0.16	195.5	0.30	246.5	0.28	299.2	0.25	310.1	0.24
33	344.0	0.06	220.5	0.12	138.2	0.23	195.5	0.18	195.5	0.19
34	238.0	0.12	203.8	0.18	171.5	0.22	212.5	0.17	228.2	0.16
35	287.9	0.06	119.1	0.12	166.5	0.09	203.8	0.07	195.5	0.08
39	72.8	0.37	138.2	0.20	151.8	0.19	181.8	0.16	212.5	0.14
40	220.5	0.12	138.2	0.46	220.5	0.29	238.0	0.26	246.5	0.25
Average....	224.3	0.12	169.6	0.19	180.3	0.18	207.8	0.17	220.3	0.17

Z unit for the ventricle (normally) would have been 196.0 instead of 220.0 as shown by the control frogs. As actually determined in the fatigued condition the threshold was 165.8 Z units. Thus instead of the rise in irritability amounting to 54.5 Z units, it amounts to 30.2 or 15.4 per cent. However, the change as expressed by the cardio-skeletal quotient percentage probably is not too high when it is realized that a change in the quotient of only about 5 per cent results in a corresponding change in ventricular Z units of over 15 per cent. In order to test further the validity of the cardio-skeletal quotient in making such estimations with a series of animals, the control animals in this investigation were used and the Z units of the ventricles were estimated from the gastrocnemius Z units, using the cardio-skeletal quotient, 0.17. The average of the Z units of the ventricles found in these estimations was 225.2. Looking then at the actual determinations the average was found to be 220.3, a difference of only 4.9 Z units or 2.2 per cent.

TABLE 2

Showing the threshold stimulus of the ventricle in fatigued frogs immediately after introduction of the electrodes and at five-minute intervals thereafter for twenty minutes: the corresponding cardio-skeletal quotients (C. S. Q.) are also shown.

EXPERIMENT NUMBER	0 MINUTES		5 MINUTES		10 MINUTES		15 MINUTES		20 MINUTES	
	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.	Z.	C.S.Q.
11	212.5	0.06	171.5	0.07	181.8	0.06	220.5	0.05	238.8	0.05
12	195.5	0.11	156.6	0.15	176.5	0.15	181.8	0.13	176.5	0.13
13	255.9	0.09	142.1	0.20	142.1	0.22	151.8	0.21	142.1	0.23
14	255.9	0.13	156.6	0.22	171.5	0.21	176.5	0.21	112.8	0.41
15	344.0	0.03	176.5	0.07	123.0	0.10	134.5	0.09	123.0	0.10
16	332.8	0.07	151.8	0.20	130.0	0.24	126.8	0.24	123.0	0.28
17	412.5	0.04	171.5	0.13	176.5	0.13	166.5	0.15	156.6	0.16
18	195.5	0.22	238.0	0.18	228.2	0.21	195.5	0.28	101.2	0.55
19	176.5	0.21	151.8	0.30	171.5	0.29	142.1	0.35	126.8	0.39
20	246.5	0.09	310.1	0.08	171.5	0.15	203.8	0.12	151.8	0.16
21	176.5	0.08	228.2	0.09	265.9	0.11	228.2	0.14	228.2	0.15
22	171.5	0.19	238.0	0.16	171.5	0.23	220.5	0.19	246.5	0.17
23	366.1	0.03	287.9	0.05	255.9	0.05	166.5	0.09	166.5	0.09
36	166.5	0.16	246.5	0.15	310.1	0.14	255.9	0.22	188.0	0.31
37	299.2	0.09	171.5	0.18	115.7	0.25	109.2	0.27	106.5	0.27
38	508.0	0.04	355.5	0.08	332.8	0.12	310.1	0.16	265.9	0.18
Average....	269.6	0.10	209.6	0.14	195.2	0.16	186.8	0.18	165.8	0.22

The increase in irritability of the heart as shown by the cardio-skeletal quotient estimated at twenty minutes after fatigue does not appear very great, but that it is definite is revealed by a more extended analysis of the cardio-skeletal quotient from the moment the electrodes were introduced. In the control animals the cardio-skeletal quotient at 0 and at five minute intervals thereafter was as follows: 0.12, 0.19, 0.18, 0.17, 0.17; in the fatigued animals it was: 0.10, 0.14, 0.16, 0.18, 0.22. In experiment 51 where the cardio-skeletal quotient was estimated for a longer period it was: 0.13, 0.12, 0.10, 0.18, 0.19, 0.23, 0.26, 0.28, 0.30, 0.34. Further evidence of this tendency of the cardio-skeletal quotient to rise in fatigued animals is shown by the average obtained in a series of frogs which were moderately fatigued, or rather the fatiguing process was only carried to the extent which the animal could voluntarily produce. In this series the cardio-skeletal quotients were as follows: at twenty minutes, 0.20; at forty minutes, 0.36; at one hour, 0.48. This tendency is shown graphically in figure 1. In plotting the curves of irritability in those experiments where the observations were carried on for more than twenty minutes, the Z units were used in place of the cardio-skeletal quotient. Curve *C* shows the average cardio-skeletal quotient in the fatigued animals. The ordinates, designated at the left of the figure, represent the units of the cardio-skeletal quotient; the abscissas, designated at the top of the

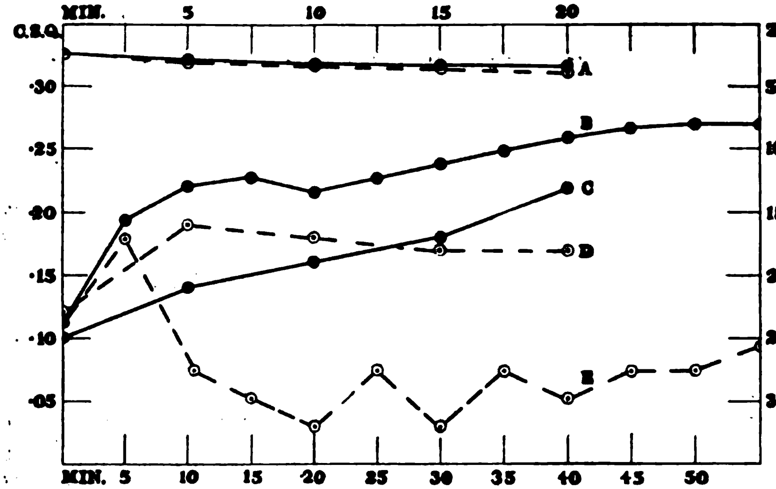

Fig. 1. Showing relative irritability of the heart in normal frogs (curves *D* and *E*), and in fatigued frogs (curves *B* and *C*).

figure, represent the time intervals. Curve *D* shows the cardio-skeletal quotient in the control animals. At *A* are plotted the curves of irritability of the gastrocnemii of the control (interrupted line) and the fatigued animals. The time intervals correspond to those of curves *C* and *D*. The ordinates, indicated at the right of the figure, represent the strength of stimulus in Z units. Curve *B* shows the irritability of the heart in experiment 46, a fatigued frog; curve *E* shows the irritability of the heart in experiment 48, a non-fatigued frog. The time intervals are designated at the bottom of the figure, the strength of stimulus in Z units is indicated at the right of the figure. These two curves, *B* and *E*, show practically the same result as does a composite curve of all the experiments but were chosen not only for that reason but because the initial stimulus of each happened to be the same, 238.0 Z units. Their curves may be taken as representative of the curve of irritability of the heart in moderately fatigued and in non-fatigued frogs. The irritability of the hearts in the fatigued animals, as shown in this series, is first low, then increases to normal and rises above normal. In animals where observations were carried on for a longer period than twenty minutes, the increase in irritability continued at least for an hour. In the control animals the irritability is at first low, rises above normal and then returns to normal where it remains for an hour or longer.

In order to see if this rise of irritability was simply due to excessive fatigue, a series of eight experiments was performed in which fatigue was produced that was not greater than the animal was capable of producing voluntarily. The cardio-skeletal quotients have been given above. Usually these experiments showed not so much depression of irritability at the beginning but rather an initial high irritability which became still greater as time went on. Experiment 46 shown in figure 1 at *B* is representative of this kind of an experiment.

All the data agree that the introduction of the electrodes into the heart muscle must produce at first a depression of irritability, since in both control and fatigued animals the curve of irritability rises during the first five minutes. In the normal animal it sinks back to a point which may be maintained for an hour or longer. In the fatigued animal it continues to rise. In other words, fatigue seems to cause the threshold of the heart to become more nearly the magnitude of that of a normal skeletal muscle. Thus in experiment 6, after an interval of twenty minutes it required only one and a half times as strong a stimulus to make the ventricle contract as it did to make the normal unfatigued gastrocnemius contract.

A further contrast was noted in the behavior of the normal hearts and those in the fatigued animals. In the former the heart would occasionally begin to beat spontaneously during the first five or ten minutes of the experiment, it would continue for a few minutes and then remain quiescent indefinitely. The fatigued hearts never showed this peculiarity during the first few minutes, but later when irritability, as shown by the strength of stimulus, had risen, it was not uncommon for it to start beating. The experiments in which spontaneous beating occurred were discarded.

The evidence obtained shows that the heart, like other tissues of the body, is affected by fatigue products. Unlike skeletal muscle, its irritability is increased rather than decreased. In the presence of large amounts of fatigue products the heart may also show a depression of irritability. This is indicated by the fact that the curve of the cardio-skeletal quotient in the extremely fatigued animals begins at a lower point than does that of the normal animals and does not reach the curve of the normal animals for about fifteen minutes. The curve indicates that when the amount of fatigue products is reduced to a certain point, then the influence is one of stimulation rather than depression. This is probably the condition in ordinary fatigue, as is shown at *B* in figure 1. Here the fatigue products could not have been so enormous as in curve *C* where the fatiguing was carried beyond the point where an animal could produce it voluntarily. In experiment 46 the animal did its own fatiguing, and it is noted that only its initial stimulus was as low as that in a normal heart; for a period of an hour it was considerably higher than the normal. This was true of all the experiments where the fatigue produced was of this moderate character. It is altogether probable that it is impossible for an animal to so fatigue itself that the resulting fatigue products would be present in sufficient quantities to *reduce* the irritability of the heart muscle. In moderate work it is conceivable that the increase in irritability of the heart has an adaptive purpose; thus with increased activity of the skeletal muscles, the heart muscle is influenced by the increase in fatigue products in the circulation and more readily responds to stimulation. Electrical measurements show this is true. Whether or not its response to chemical stimuli is also increased as a result of fatigue was not studied in this series. The continuation of the rapid beating of the heart following exercise may in part be explained by its increase of irritability due to the presence of fatigue products. It is also conceivable that continued fatigue as in overwork may give rise to cardiac disturbances that are the result of increased irritability of the heart muscle.

The evidence obtained seems to justify the following conclusions:

1. The heart, like other tissues of the body, is affected by the products of fatigue.

2. Extreme fatigue, i.e., carried to a point impossible of attainment by voluntary efforts, causes a depression of the electrical irritability of the heart, followed by a rise of irritability which may continue for some time.

3. Moderate fatigue, i.e., carried to a point possible of attainment by voluntary efforts, causes a marked rise in the irritability of the heart muscle.

BIBLIOGRAPHY

(1) **Mendenhall**: This Journal, 1917, xliii, 202.
(2) **Mendenhall**: Loc. cit., 195.
(3) **Martin**: The measurement of induction shocks, New York, 1912.
(4) **Mendenhall**: Loc. cit., 200.

THE OUTPUT OF EPINEPHRIN IN SHOCK[1]

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In most of the experiments the classical method of producing experimental shock, exposure with or without manipulation of the intestines, was adopted. "Manipulation" in our experiments included displacement, handling, pinching firmly between the fingers and thumb, flicking with the fingers, tapping with such objects as the handle of a scalpel, etc. Compression of the inferior vena cava till a condition of permanently low blood pressure was induced was also employed, since this method has been extensively used of late in the study of shock. We do not, however, consider this the most suitable method when the behavior of the adrenal is to be studied because of the possibility that a more or less prolonged period of passive congestion of the gland may be a disturbing factor. In one or two experiments a state of continued low blood pressure was induced by hemorrhage and in one experiment by the injection of "peptone." It is not to be imagined that exactly the same physiological changes are brought about when the blood pressure is permanently lowered in all these different ways. On the contrary, the condition produced by peptone injection obviously differs in important features from the condition which follows copious hemorrhage and this from the condition or conditions produced by the exposure of the intestines or partial occlusion of the cava, which are perhaps more properly termed experimental shock.

It was in order to increase the chance of finding a procedure which would grossly alter the rate of output of epinephrin that so many and such different methods were employed. It is all the more notable that in none of the experiments was any evidence obtained that the rate of output was sensibly modified as compared with the initial rate. This result constitutes a new and striking illustration of the fact dwelt upon in previous papers (1) that the output of epinephrin is relatively stable and not easily influenced experimentally.[2]

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[1] About six months ago I was asked to become a member of the Sub-Committee on Shock (Medical Division of the National Research Council), the work more particularly assigned being an investigation of the output of epinephrin in that condition. The investigation has been made jointly by Doctor Rogoff and myself. A report summarizing the results presented in this paper was sent to the Committee September 28, 1918—G. N. S.

The range of epinephrin output per kilogram per minute is, as determined by us, surprisingly narrow, not only in animals of the same species but in different groups. We have had the opportunity of determining the output in a baboon, weighing 2.51 kgm. It worked out at 0.0002 mgm. per kgm. per minute. The average found by us in cats, as assayed by intestine and uterus segments, is 0.00025 mgm. per kgm. per minute, and it is not very different in the dogs examined. Statements in the literature (6) to the effect that in shock conditions the output is much increased, even it may be to more than thirty-two times the initial output, are based upon faulty experimentation, the method of bio-assay adopted not being properly applied. The tracings accompanying the paper cited do not indicate in what manner the test objects (rabbit intestine "strips," meaning probably segments) were subjected to the action of the bloods. No quantitative estimations of the amount of epinephrin liberated per unit of time either before or after shock was induced are given. Nor is there any statement of the concentrations of epinephrin in the blood from which such estimations could be made. We are unable to follow the statement that shock can be induced without any lowering of blood pressure and "that in cases of shock accompanied by high blood pressure, the blood contains relatively a large amount of epinephrin." Some writers have asserted that in shock the store of epinephrin in the suprarenals is diminished. Apart from the fact that the methods used for the assay have ordinarily been inadequate, and that account has not been taken of the effect of anesthetics upon the store, there is no necessary relation between diminished store and increased output, as we have more than once pointed out (7).

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[2] The fact that the output of epinephrin per minute remains practically the same with very different rates of blood flow through the gland is of itself sufficient to invalidate the contention of Popielski (2) against the existence of secretory nerves for the adrenals. He asserts that the result of stimulation of the splanchnics on the liberation of epinephrin is merely due to washing out already accumulated epinephrin through the increased blood flow. Since we have shown that increased output does not as a matter of fact accompany increased blood flow, this argument falls to the ground. It is astonishing that it should ever have been put forward. The complete abolition of the output of epinephrin by section of the nerves of the gland is certainly not accompanied by any marked diminution in the blood flow. We have not noticed that it is materially altered. And Burton-Opitz and Edwards (3) saw no definite increase in the adrenal blood flow during stimulation of the peripheral end of the splanchnic when a change of general arterial pressure was prevented. Popielski's statement that manipulation of the adrenal does not liberate epinephrin unless the gland is afterwards flushed out with blood, as by stimulating the splanchnics, is manifestly erroneous, as abundantly demonstrated in our own work (4), (5).

A few of the protocols and some of the tracings used for the epinephrin assay in our experiments follow. The epinephrin was assayed by the method introduced by one of us (rabbit intestine segments) (8). In most cases the rabbit uterus segment was also employed to corroborate and sometimes to render more delicate the assay made on the intestine. As we have pointed out in previous papers, it is essential in order to obtain the best results from these methods of bio-assay, that numerous tests should be made with each blood. As a general rule it is advantageous to approximate to the epinephrin concentration in the blood specimen by determining a concentration of adrenalin which is definitely greater and one which is definitely less; then narrowing the interval between the higher and lower concentrations by a sufficient number of observations. It is then not difficult to obtain a concentration of adrenalin which gives a tracing approximately the same as that of the blood. This, however, is not always necessary in order to make a sufficiently close assay, if the higher and lower concentrations are not too far apart.

Condensed protocol

Dog 9. Male. Weight 6.6 kgm.
9.30 a.m. 50 mgm morphine hypodermically
10.45 a.m. Etherized. Put cannulae in trachea, carotid and jugular. Obtained specimen of indifferent blood from jugular
11.05 to 11.30 a.m. Made cava pocket,[3] tying abdominal aorta near the bifurcation

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[3] In all of the experiments the cava pocket was made in a similar manner. The renal vessels and all veins entering the segment of cava between the liver and the point of insertion of the cannula were tied, leaving a pouch into which only the adrenal veins entered. The abdominal aorta was tied near the bifurcation. The cannula was inserted into the lower end of the cava and after pressing the blood out of the segment of cava the pocket was completed by a clamp, just beneath the liver immediately before collection of adrenal blood. After collecting each pair of specimens a fresh cannula was inserted into the pocket.

11.30 a.m. Arterial blood pressure 90 mm. of mercury. Now collected adrenal vein blood from the pocket
1st specimen 4.75 grams in 30 seconds (9.6 grams per minute)
2nd specimen 9.6 grams in 60 seconds (9.6 grams per minute)
11.35 a.m. Exposed intestines, with occasional manipulation
12.30 p.m. Blood pressure 68 mm. of mercury
12.32 p.m. Collected adrenal blood from pocket
3rd specimen, 2.35 grams in 30 seconds (4.7 grams per minute)
4th specimen, 7.3 grams in 90 seconds (4.7 grams per minute)
12.40 p.m. Replaced intestines in abdomen and fastened abdomen
12.45 p.m. Blood pressure 70 mm. of mercury
1.10 p.m. Blood pressure 80 mm. of mercury. Again exposed intestines
1.25 p.m. Blood pressure 60 mm. of mercury
1.45 p.m. Blood pressure 55 mm. of mercury
1.50 p.m. Collected adrenal blood from pocket
5th specimen, 2.3 grams in 60 seconds (2.3 grams per minute)
6th specimen, 6.8 grams in 180 seconds (2.3 grams per minute)
Combined weight of adrenals, 0.848 gram.

The epinephrin assay was made on the 2nd, 4th and 6th adrenal blood specimens. As in all this work, a specimen was collected immediately before each of these (1st, 3rd and 5th), so as to wash the cava segment completely free from any blood entering it during the manipulation connected with the clipping off of the pocket. The flow was not interrupted at all between the collection of the 1st and 2nd, 3rd and 4th or 5th and 6th specimens. After each pair of specimens had been obtained a fresh cannula was inserted into the cava so as to be ready for the next collection. In figures 1 to 6 are reproduced some of the tracings used for the assay. Those of the 6th specimen are given more completely than the others so as to illustrate once for all the manner in which the concentration of epinephrin in the blood is approximated to.

Figure 1 shows that the 2nd specimen, collected before the induction of shock, is notably weaker than the 4th, collected one hour after exposure of the intestines with a blood pressure of 68 mm., and that the 4th specimen is weaker than the 6th, collected two and one-quarter hours after exposure of the intestines, with a blood pressure of 55 mm. or less. This figure is reproduced to emphasize the fact that a qualitative comparison of tracings can never be used to determine whether a change in the rate of output of epinephrin has occurred, a point habitually neglected by some writers on this subject.

Although the much greater inhibition of the intestine segment produced by the 6th specimen than by the 2nd might suggest to a reader unfamiliar with these methods of assay that the output had been much increased after shock, the assay proved that as a matter of fact there was no change. Figure 2 shows that the 6th specimen (observation 16) is much stronger than 1:3,750,000 adrenalin (observation 12), and stronger than 1:2,500,000 (observation 14). The tracings reproduced in figure 3 demonstrate that the 6th specimen (observation 48) is stronger than 1:1,875,000 adrenalin (observation 50). Figure 3-A shows that it does not differ greatly from 1:1,250,000. The 2nd specimen is shown in figure 4 to be stronger than adrenalin 1:8,750,000. A pair of observations made immediately thereafter (not reproduced) proved that the 2nd specimen was stronger than adrenalin 1:7,500,000. A pair of observations made immediately before those in figure 4 indicated that the concentration of epinephrin in the 2nd specimen did not differ much from 1:6,250,000, and this was confirmed by later observations (not reproduced) on the same intestine segment.

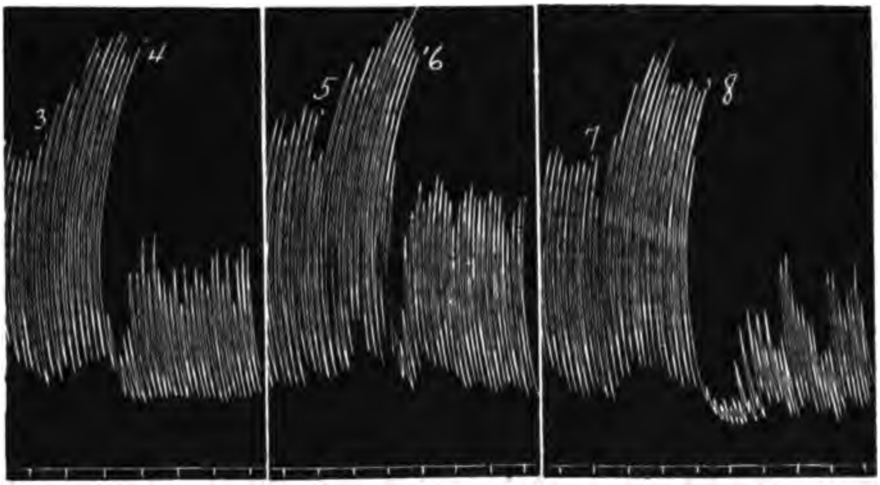

Fig. 1. Intestine tracings. Bloods from dog 9. At *3*, *5* and *7* Ringer was replaced by jugular blood and this was replaced at *4* by the 4th adrenal blood specimen (collected after 1 hour of exposure of intestines, blood pressure 68 mm. of mercury); at *6* by the 2nd adrenal blood specimen (collected before exposure of intestines, blood pressure 90 mm. of mercury) and at *8* by the 6th adrenal blood specimen (collected after 2 hours and 20 minutes exposure of intestines, blood pressure 55 mm. of mercury). All the bloods were diluted with 3 volumes of Ringer. As in all the tracings, time is marked in half-minutes. (Reduced to one-half.)

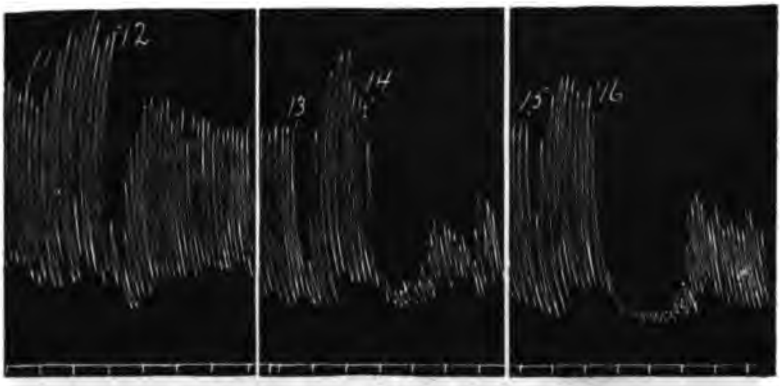

Fig. 2. Intestine tracings. Bloods from dog 9. At *11*, *13* and *15* Ringer was replaced by jugular blood and this was replaced at *12* by jugular blood to which had been added adrenalin to make a concentration of 1: 3,750,000; at *14* by jugular blood with adrenalin added to make a concentration of 1: 2,500,000 and at *16* by the 6th adrenal blood specimen, which is stronger than 1:2,500,000 (from other observations twice as strong). The difference between 14 and 16 appears relatively small simply because the inhibition at 14 is already so great. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

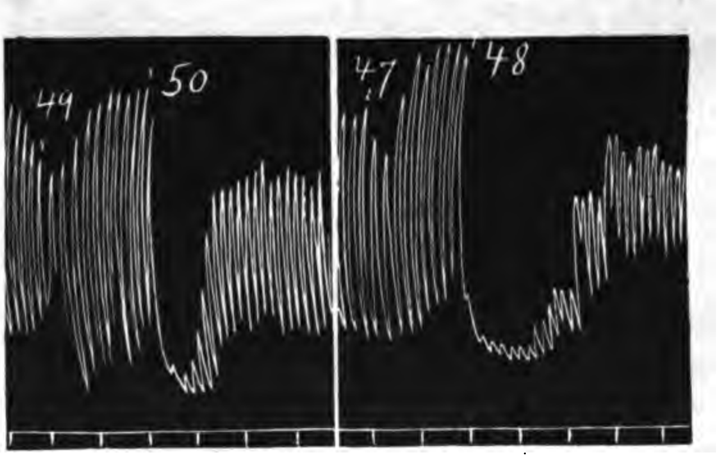

Fig. 3. Intestine tracings. Bloods from dog 9. At *47* and *49* Ringer was replaced by jugular blood and this was replaced at *48* by the 6th adrenal blood specimen and at *50* by jugular blood to which was added adrenalin to make a concentration of 1: 1,875,000. The bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

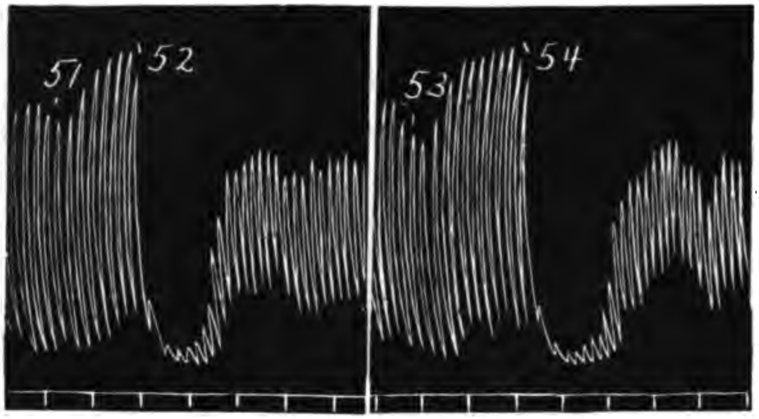

Fig. 3-A. Intestine tracings. Bloods from dog 9. At *51* and *53* Ringer was replaced by jugular blood and this was replaced at *52* by jugular blood with adrenalin added to make a concentration of 1: 1,250,000 and at *54* by the 6th adrenal blood specimen. The bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

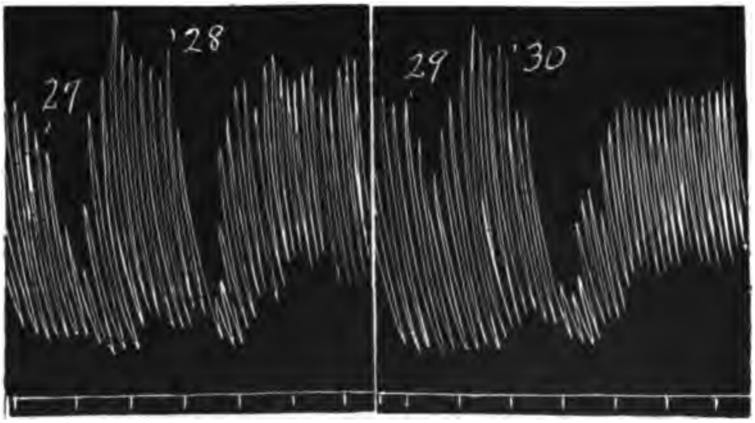

Fig. 4. Intestine tracings. Bloods from dog 9. At *27* and *29* Ringer was replaced by jugular blood and this was replaced at *28* by jugular blood to which was added adrenalin to make a concentration of 1: 8,750,000 and at *30* by the 2nd adrenal blood specimen (collected before exposure of intestines). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

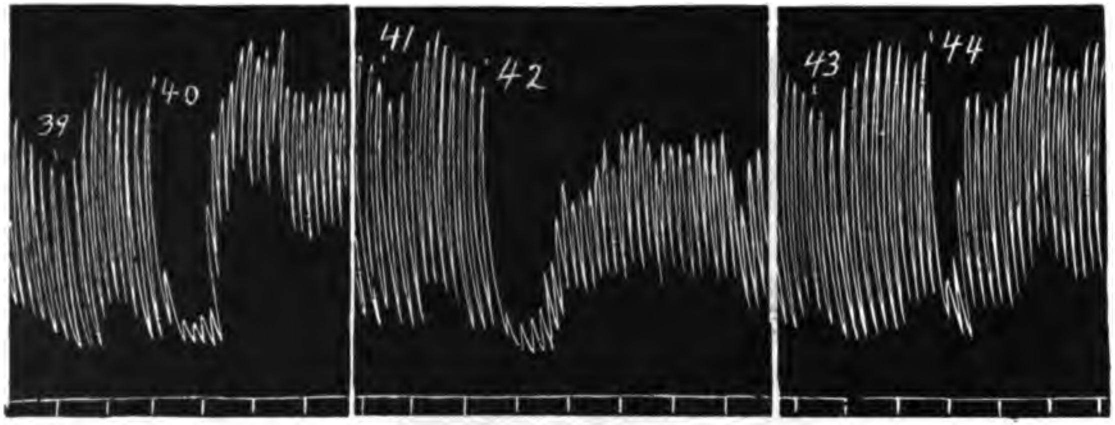

Fig. 5. Intestine tracings. Bloods from dog 9. At *39*, *41* and *43* Ringer was replaced by jugular blood and this was replaced at *40* by the 4th adrenal blood specimen (collected after 1 hour exposure of intestines, blood pressure 68 mm. of mercury); at *42* by jugular blood to which was added adrenalin to make a concentration of 1: 2,500,000 and at *44* by jugular blood with adrenalin added to make a concentration of 1: 3.750,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to four-fifths.)

In figure 5 it is demonstrated that the 4th adrenal specimen (observation 40) is decidedly weaker than 1: 2,500,000 (observation 42) and somewhat stronger than 1: 3,750,000 (observation 44). It was confirmed by two other pairs of observations (not reproduced), one just before and the other just after those recorded in figure 5, that the 4th specimen was not much different from 1: 3,750,000, probably a little stronger.

In figure 6 are given samples of uterus tracings showing that the 2nd adrenal specimen produces a greater increase of tone than the indifferent (jugular) blood; the 4th specimen a greater increase than the 2nd and the 6th a much greater increase than the 4th. Again it must be pointed out that the only conclusion which could be drawn from a comparison of the curves in figure 6, taken by themselves, would be that the concentration of epinephrin in the 6th specimen was greater than that in the 4th and the concentration in the 4th greater than that in the 2nd. It would be impossible to give any quantitative value to the differences. We know that in fact the 6th specimen was about three times as strong as the 4th and the 4th less than twice as strong as the 2nd. But this information is only obtainable by a systematic and rather laborious assay. Yet some writers from mere inspection of a few tracings revealing far less striking differences than these shown in figure 6, without any

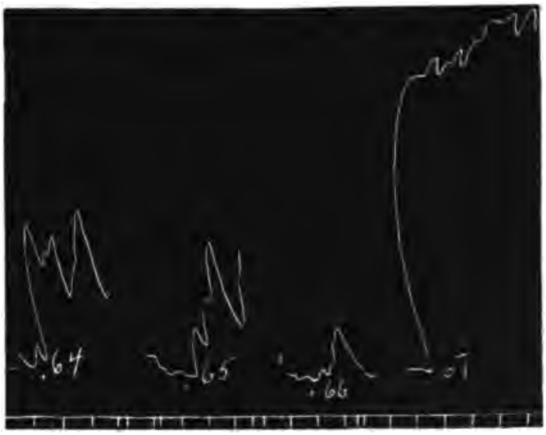

Fig. 6. Uterus tracings. Bloods from dog 9. At *64* Ringer was replaced by the 4th adrenal blood specimen; at *65* by the 2nd adrenal blood specimen; at *66* by jugular blood and at *67* by the 6th adrenal blood specimen. All the bloods were diluted with 2 volumes Ringer. (Reduced to one-half.)

attempt at quantitative estimation, have ventured to draw far reaching conclusions as to the influence of this or that factor in markedly increasing the rate of output of epinephrin. It may be safely assumed that when a paper does not give the amount of epinephrin liberated per unit of time, before and after the operation of the factor supposed to influence the rate of liberation but merely concludes that the rate has been greatly increased or greatly diminished, it does not contain any real evidence on the question.

Taking the concentration of epinephrin in the second specimen from dog 9 as 1:6,250,000 we get an output of 0.0015 mgm. per minute for the dog, or 0.00023 mgm. per kgm. of body weight per minute. Taking the 4th specimen at 1:3,500,000 we get 0.0013 mgm. per minute for the dog, or 0.0002 mgm. per kgm. per minute. Taking the concentration in the 6th specimen as 1:1,250,000 we get 0.0018 mgm. per minute for the dog, or 0.00027 mgm. per kgm. per minute.

Condensed protocol

Dog 6. Weight 6.2 kgm.
11.00 a.m. Morphine 25 mgm. hypodermically
1.00 p.m. Etherized. Put cannulae in trachea, carotid and jugular. Made cava pocket
1.50 p.m. Blood pressure 154 mm. of mercury
1.55 p.m. Obtained indifferent blood from jugular. Now collected adrenal blood from the pocket
1st specimen, 7.6 grams in 45 seconds (11.5 grams per minute)
2nd specimen, 22.9 grams in 120 seconds (11.5 grams per minute)
Blood pressure during collection of these specimens 123 mm. of mercury
2.00 p.m. Exposed intestines; occasional manipulation
2.07 p.m. Blood pressure 85 mm. of mercury
2.35 p.m. Blood pressure 66 mm. of mercury
2.55 p.m. Blood pressure 63 mm. of mercury. Collected adrenal blood from pocket
3d specimen, 7.4 grams in 60 seconds (7.4 grams per minute)
4th specimen, 16.0 grams in 150 seconds (6.4 grams per minute)
Combined weight of adrenals 0.905 gram.

In this experiment the epinephrin assay showed that the 2nd adrenal blood specimen (taken before manipulation of the intestines was begun, with a blood pressure of 123 mm. of mercury) had a concentration of of 1:6,500,000. The output per minute for the dog was 0.0018 mgm., or 0.0003 mgm. per kgm. of body weight per minute. The 4th specimen collected nearly an hour after manipulation was begun, with blood pressure of 63 mm. of mercury, had a concentration of 1:3,500,000, corresponding to an output per minute for the dog of 0.0018 mgm., or 0.0003 mgm. per kgm. per minute. A small sample of the tracings used in the assay is reproduced in figure 7, which shows that the 4th specimen (observation 17) had a concentration decidedly greater than 1:4,000,000 (observation 19), but somewhat less than 1:3,000,000 (observation 21). This was confirmed by other observations.

In a third experiment on a dog, weighing 7.25 kgm. (dog 7), a specimen of adrenal blood collected before exposure of the intestines, with a blood pressure of 113 mm. of mercury, had a concentration of epinephrin of 1:1,800,000, giving an output of 0.0018 mgm. per minute for the dog, or 0.00025 mgm. per kgm. per minute. A specimen, collected two hours after exposure of the intestines, was assayed at 1:1,250,000 epinephrin, corresponding to an output per minute for the dog of 0.0014 mgm., or 0.0002 mgm. per kgm. per minute. The blood pressure ten minutes before collection of this specimen was 72 mm. of mercury and at the end of collection it was 48 mm. of mercury. The flow during collection of the last specimen was probably too small, considering that

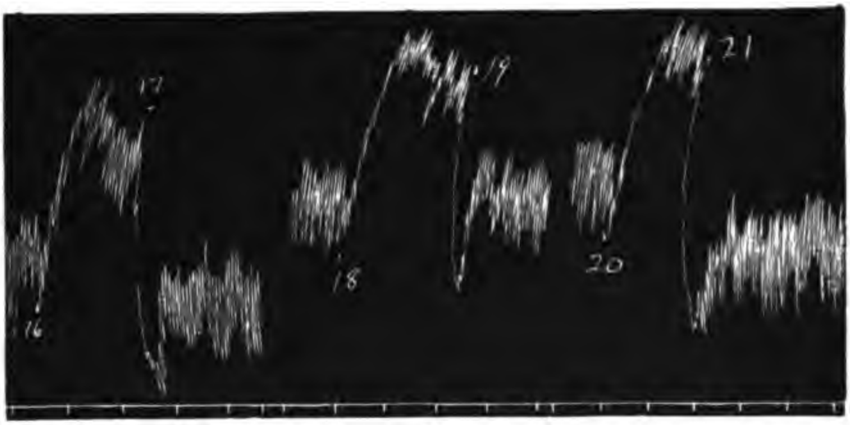

Fig. 7. Intestine tracings. Bloods from dog 6. At *16*, *18* and *20* Ringer was replaced by jugular blood and this was replaced at *17* by the 4th adrenal blood specimen (collected after 1 hr. exposure of intestines, blood pressure 63 mm. of mercury); at *19* by jugular blood to which was added adrenalin to make a concentration of 1:4,000,000 and at *21* by jugular blood with adrenalin added to make a concentration of 1:3,000,000. All the bloods were diluted with 2 volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to four-sevenths.)

the concentration was approaching the possible maximum to allow the calculated output quite to equal the output before shock was induced.

Condensed protocol

Dog. 2.	Male. Weight 7.3 kgm.
9.00 a.m.	Morphine 50 mgm. hypodermically
10.50 a.m.	Etherized. Put cannulae into trachea, carotid and jugular. Obtained indifferent blood from jugular
11.25 a.m.	Started artificial respiration
11.45 a.m.	Loose ligature placed around inferior cava, in thorax. Made cava pocket
12.40 p.m.	Blood pressure 140 mm. of mercury.
12.50 p.m.	Blood pressure 100 mm of mercury. Now collected adrenal blood from the pocket
	1st specimen, 7.85 grams in 60 seconds (7.85 grams per minute)
	2nd specimen, 16.1 gram in 120 seconds (8.0 grams per minute)
1.00 p.m.	Partial occlusion of cava with occasional release, practiced at intervals for one and one-half hours. During this period the blood pressure gradually fell to 68 mm. of mercury at 2.30 p.m.
2.45 p.m.	Collected adrenal blood from pocket
	3rd specimen, 4.2 grams in 60 seconds (4.2 grams per minute)
	4th specimen, 11.35 grams in 180 seconds (3.8 grams per minute)
	5th specimen, 8.85 grams in 180 seconds (3.0 grams per minute)

Combined weight of adrenals 1.2 grams.

The second adrenal blood specimen (from dog 2) collected before occlusion of the cava, with blood pressure 105 mm. of mercury, was assayed at 1:6,500,000 corresponding to an output of 0.0012 mgm. per minute for the animal, or 0.00017 mgm. per kgm. per minute. The 4th specimen, collected after shock had been induced, contained 1:2,700,000 epinephrin, giving an output of 0.0014 mgm. per minute for the dog, or 0.00019 mgm. per kgm. per minute. The 5th specimen, collected with a blood pressure of 68 mm. of mercury, had a concentration of 1:2,100,000 corresponding to an output of 0.0014 mgm. per minute for the animal, or 0.00019 mgm. per kgm. per minute.

Condensed protocol

Dog 1.	Female. Weight 11.3 kgm.
9.00 a.m.	Morphine 100 mgm. hypodermically
10.45 a.m.	Etherized. Put cannulae in trachea, carotid and jugular. Made cava pocket

11.30 a.m. Blood pressure 100 mm. of mercury. Now collected adrenal blood from pocket
1st specimen, preliminary specimen
2nd specimen, 17.4 grams in 30 seconds (34.8 grams per minute)
11.45 a.m. Bled from carotid (about 250 cc. blood withdrawn). Blood pressure just after hemorrhage, 60 mm. of mercury. Collected a preliminary specimen, then
3d specimen, 9.5 grams in 60 seconds
11.50 a.m. Closed abdomen with clamps
12.00 to 12.30 p.m. Pulse feeble and rapid; respirations very rapid (180 per minute)
1.50 p.m. Collected adrenal blood from pocket
4th specimen, 3.35 grams in 30 seconds (6.7 grams per minute)
5th specimen, 11.45 grams in 150 seconds (4.6 grams per minute)
Blood pressure just after collection of 5th specimen 40 to 50 mm. of mercury
Combined weight of adrenals 1.8 grams.

In this animal the 2nd adrenal blood specimen, obtained before hemorrhage, with the blood pressure at 100 mm. of mercury, was assayed at 1: 18,000,000 epinephrin. The rate of flow from the adrenals was large although no unligated small vein connected with the cava pocket was found *post mortem.* This accounts for the exceptionally low concentration of epinephrin. The output of epinephrin per minute for the animal for this specimen was 0.002 mgm. or 0.00018 mgm. per kgm. of body weight per minute. The 3rd specimen, collected immediately after hemorrhage, with the blood pressure at 60 mm. of mercury, had a concentration of 1: 3,300,000 of epinephrin, corresponding to an output of 0.0029 mgm. per minute for the dog, or 0.00025 mgm. per kgm. per minute. The 5th adrenal blood specimen, collected two hours after the hemorrhage, the blood pressure being 50 mm. of mercury at the beginning and 40 mm. of mercury at the end of collection, had a concentration of epinephrin of 1:1,500,000. This corresponds to an output of 0.003 mgm. per minute for the animal, or 0.00025 mgm. per kgm. per minute. Samples of the tracings used for the assays are reproduced in figures 8 to 12.

Figure 8 shows that at this stage the second adrenal specimen (observation 3) caused no definite inhibition of the intestine segment, which was not sensitive enough to detect the concentration of epinephrin contained in it, although later on it was assayed at 1: 18,000,000. The 3rd specimen caused a good inhibition (observation 5). It was assayed in other observations at 1:3,300,000. This is an excellent illustration of the danger of drawing quantitative conclusions from qualitative experiments. No doubt the inhibition in observation 5 was produced by a concentration of epinephrin five times as great as that which failed to cause inhibition in observation 3. But similar observations have sometimes been erroneously interpreted as indicating that a great "outburst" of epinephrin must have occurred when the specimen giving the positive reaction was being collected. In the present instance, as the assay showed, the rate of output of epinephrin was practically the same for both specimens. Figure 9 demonstrates that the 2nd adrenal specimen contained enough epinephrin to be detected and approximately assayed by the same rabbit intestine segment when it had become more sensitive to epinephrin. The concentration is obviously less than 1:16,500,000 (observation 43) and greater than 1:20,000,000 (observation 45). These observations were confirmed by others and the concentration worked out at approximately 1:18,000,000.

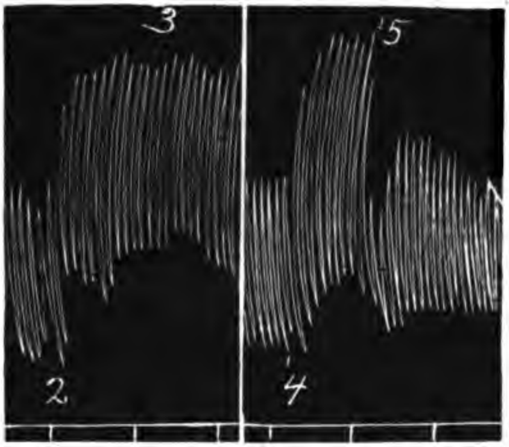

Fig. 8. Intestine tracings. Bloods of dog 1. At *2* and *4* Ringer was replaced by indifferent blood and this was replaced at *3* by the 2nd adrenal blood specimen (collected before hemorrhage, blood pressure 100 mm. of mercury) and at *5* by the 3rd adrenal blood specimen (collected a few minutes after hemorrhage, blood presure 60 mm. of mercury). The bloods were diluted with 3 volumes Ringer. (Reduced to two-thirds.)

In figure 10 it is shown that the concentration of the 3rd adrenal specimen is slightly greater than 1:3,400,000. This was confirmed by other observations. It was taken finally at 1:3,300,000. Figure 11 shows that the concentration of the 5th specimen is not very different from 1:1,650,000 and figure 12, that it is considerably less than 1:1,250,000. It was finally taken as 1,500,000.

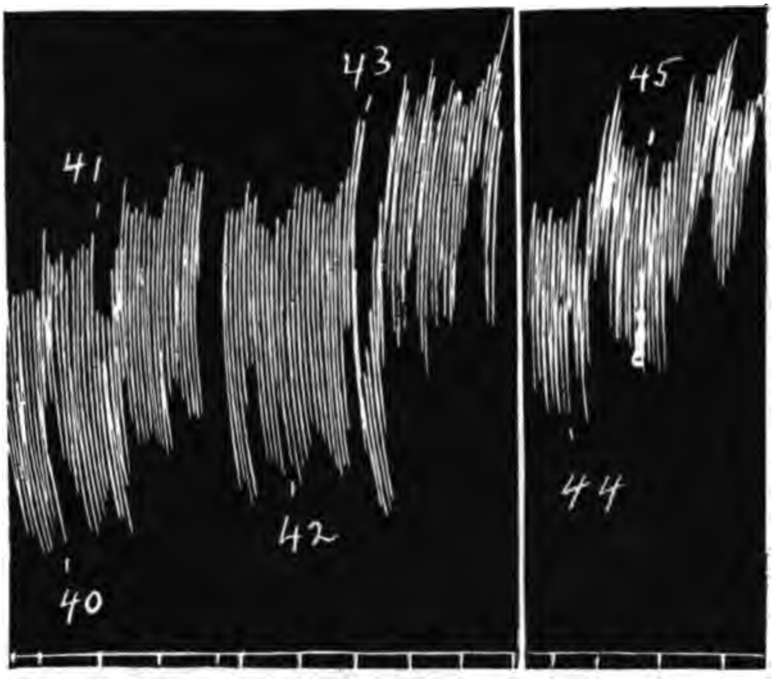

Fig. 9. Intestine tracings. Bloods from dog 1. At *40*, *42* and *44* Ringer was replaced by indifferent blood and this was replaced at *41* by the 2nd adrenal blood specimen (collected before hemorrhage); at *43* by indifferent blood to which was added adrenalin to make a concentration of 1: 16,500,000 and at *45* by indifferent blood, with adrenalin added to make a concentration of 1: 20,000,000 All of the bloods were diluted with an equal volume of Ringer (the adrenalin bloods after adding the adrenalin).

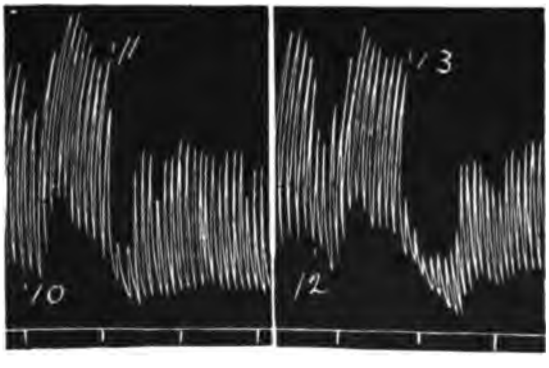

Fig. 10. Intestine tracings. Bloods from dog 1. At *10* and *12* Ringer was replaced by indifferent blood and this was replaced at *11* by indifferent blood to which was added adrenalin to make a concentration of 1: 3,400,000 and at *13* by the 3rd adrenal blood specimen (collected a few minutes after hemorrhage). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to two-thirds.)

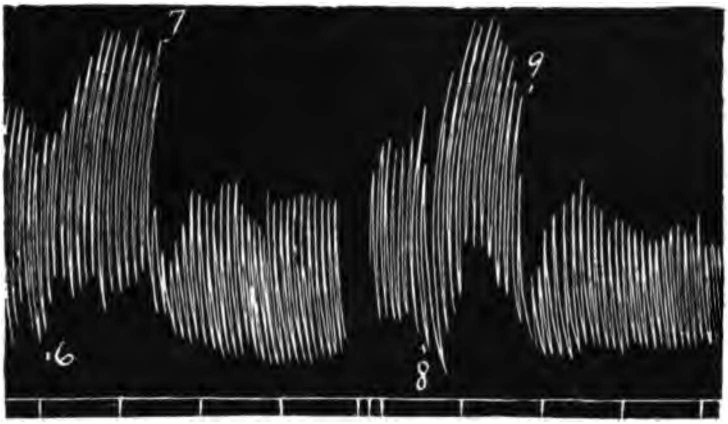

Fig. 11. Intestine tracings. Bloods from dog 1. At *6* and *8* Ringer was replaced by indifferent blood and this was replaced at *7* by the 5th adrenal blood specimen (collected 2 hours after hemorrhage, blood pressure 40 to 50 mm. of mercury) and at *9* by indifferent blood to which was added adrenalin to make a concentration of 1:1,650,000. The bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to two-thirds.)

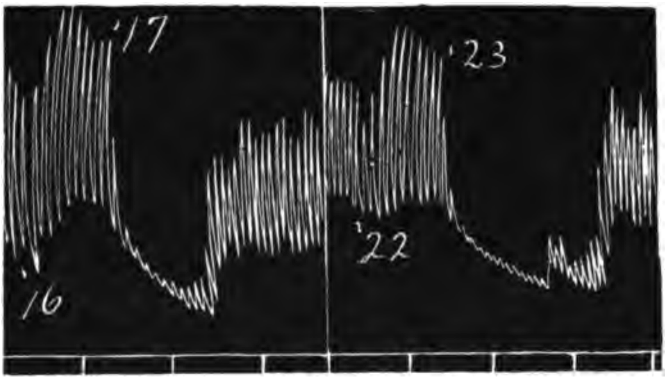

Fig. 12. Intestine tracings. Bloods from dog 1. At *16* and *22* Ringer was replaced by indifferent blood and this was replaced at *17* by the 5th adrenal blood specimen and at *23* by indifferent blood to which was added adrenalin to make a concentration of 1:1,250,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

Condensed protocol

Cat 4. Male. Weight 3.255 kgm. Urethane anesthesia.

3.00 p.m. Put cannulae in trachea, carotid and femoral vein. Obtained indifferent blood from femoral vein. Started artificial respiration. Made cava pocket

3.40 p.m. Blood pressure before opening abdomen 120 mm. of mercury. Collected adrenal blood from the pocket

1st specimen, 4.85 grams in 20 seconds (14.5 grams per minute)

2nd specimen, 6.65 grams in 30 seconds (13.3 grams per minute)

3d specimen, 6.4 grams in 30 seconds (12.8 grams per minute)

Blood pressure at beginning of collection of 3d specimen, 104 mm. of mercury

3.45 p.m. Blood pressure after collection of 3d specimen, 70 mm. of mercury. Closed abdomen with clips

3.50 p.m. Blood pressure 105 mm. of mercury

Injected 1½ grams Witte's peptone (in 10 per cent solution) *via* external jugular vein

Blood pressure at end of peptone injection, 70 mm. of mercury

4.10 p.m. Collected adrenal blood from the pocket

4th specimen, 3.65 grams in 30 seconds (7.3 grams per minute)

5th specimen, 9.95 grams in 120 seconds (5.0 grams per minute)

6th specimen, 11.20 grams in 180 seconds (3.7 grams per minute)

Blood pressure after collection of 6th specimen, 60 mm. of mercury

4.17 p.m. Obtained indifferent blood from external jugular vein. (Label this "peptone indifferent blood")

4.40 p.m. Collected adrenal blood from pocket

7th specimen, 0.72 gram in 60 seconds

8th specimen, 0.68 gram in 60 seconds

Blood pressure during collection of 8th specimen, 28 mm. of mercury

Combined weight of adrenals 0.60 gram.

The 3rd, 5th and 8th adrenal blood specimens from cat 4 were carefully assayed, that is, one specimen collected before injection of peptone and two specimens after injection. For indifferent blood the "peptone blood" collected from the jugular was used in the assay of the 5th and 8th specimens. It was tested, however, against the indifferent blood obtained before injection of peptone and found not to differ materially from it as regards its action on the segments. The 3rd adrenal specimen assayed at 1:14,000,000 to 1:15,000,000 epinephrin, corresponding to an output of 0.0009 mgm. per minute for the animal, or 0.00027 mgm. per kgm. per minute. The blood pressure was 104 mm. of mercury at the beginning of collection of the 3rd specimen. The relatively low concentration is associated with a high rate of flow through the adrenals. No leak into the cava pocket from any other source could be detected *post mortem*. The 5th adrenal specimen, taken twenty minutes after injection of the peptone, with blood pressure about 60 mm. of mercury, had a concentration of 1:6,500,000, giving an output of 0.0008 mgm. of epinephrin per minute for the cat, or 0.00025 mgm. per kgm. of body weight per minute. The 8th specimen was assayed at 1:850,000, giving an output of 0.0008 mgm. epinephrin per minute for the cat, or 0.00025 mgm. per kgm. per minute. Although the concentration in this specimen approached the possible maximum concentration, owing to the small blood flow through the adrenals, associated with the low blood pressure at the time of collection (28 mm. of mercury), the curves obtained permitted a good assay. The results were confirmed on the uterus segments. Some of the intestine segment tracings are reproduced in figures 13 to 16. Figure 13 indicates that the concentration of the 3rd specimen is somewhat less than 1: 14,000,000. In other tracings it was shown that 1: 16,500,000 was markedly weaker than the 3rd specimen, while 1: 14,000,000 was only slightly stronger. In figure 14 it is demonstrated that the 5th speci-

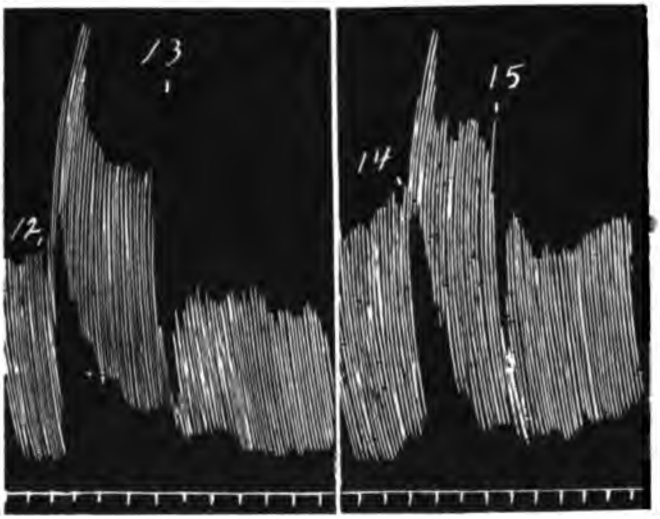

Fig. 13. Intestine tracings. Bloods from cat 4. At *12* and *14* Ringer was replaced by femoral vein blood and this was replaced at *13* by femoral vein blood to which was added adrenalin to make a concentration of 1: 14,000,000 and at *15* by the 3rd adrenal blood specimen (collected before injection of peptone, blood pressure 104 mm. of mercury). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

men was weaker than 1: 5,700,000. Other tracings showed that it was stronger than 1:7,000,000. In figures 15 and 16 are reproduced some of the tracings used to assay the 8th specimen. Figure 15 shows that 1: 1,400,000 (observation 55) was much too weak for the specimen and that 1: 700,000 (observation 57) was too strong. From figure 16 it is clear that the specimen was stronger than 1:1,000,000.

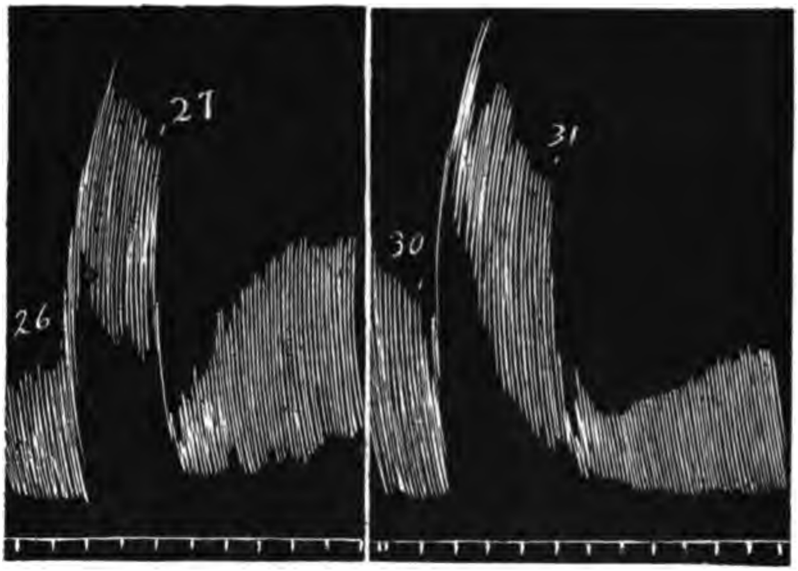

Fig. 14. Intestine tracings. Bloods from cat 4. At *26* and *30* Ringer was replaced by jugular blood and this was replaced at *27* by the 5th adrenal blood specimen (collected 20 minutes after injection of peptone) and at *31* by jugular blood to which was added adrenalin to make a concentration of 1: 5,700,000. All the bloods were diluted wih 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

The results of this investigation may be summed up in a sentence. In none of our experiments has evidence been obtained that any sensible change occurs in the rate of output of epinephrin from the adrenals in conditions of continued low blood pressure.

The only way in which we have hitherto been able to bring about an unequivocal increase in the epinephrin output (apart, of course, from that due to stimulation of the peripheral end of the splanchnic

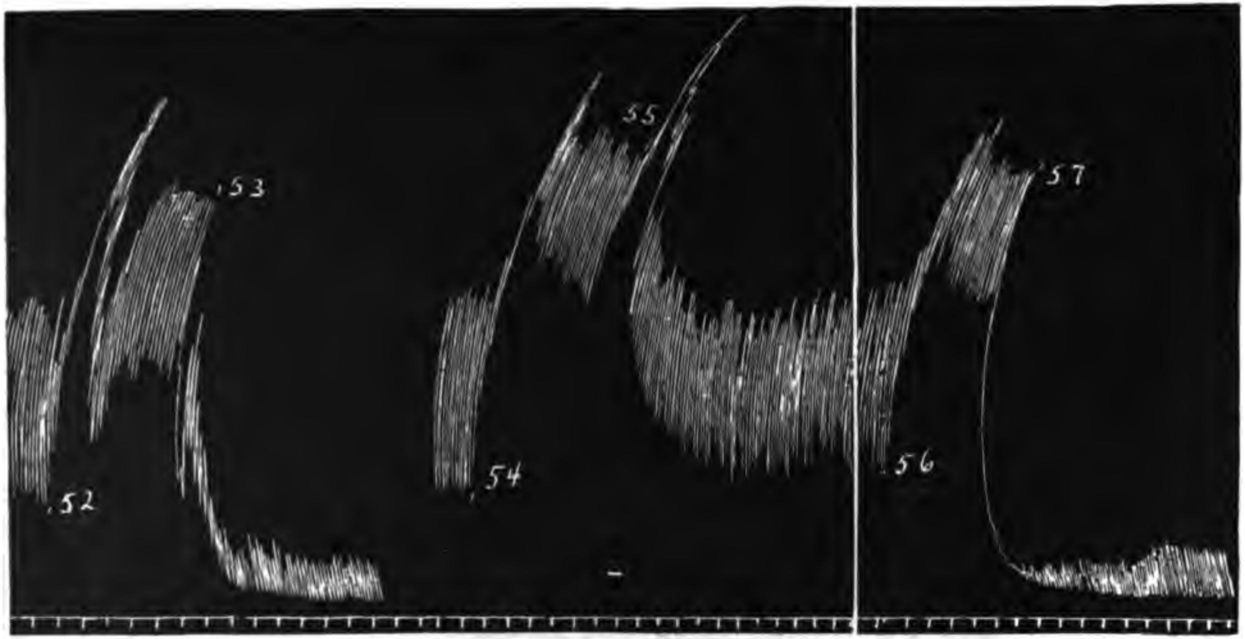

Fig. 15. Intestine tracings. Bloods from cat 4. At *52*, *54* and *56* Ringer was replaced by jugular blood and this was replaced at *53* by the 8th adrenal blood specimen (collected ¾ hour after peptone injection, blood pressure 28 mm. of mercury); at *55* by jugular blood to which was added adrenalin to make a concentration of 1 : 1,400,000 and at *57* by jugular blood with adrenalin added to make a concentration of 1 : 700,000. All the bloods were diluted with 6 volumes Ringer (the adrenalin bloods after adding the adrenalin.) (Reduced to two-thirds.)

nerve) is by the administration of strychnine or the intravenous injection of small quantities of concentrated solutions of salts (sodium carbonate), which cause a general excitation of the spinal cord. We purposely produced such an excitation in the hope of stimulating among other centers that which is associated with the secretion of epinephrin and is situated in the upper part of the thoracic cord (9). For this reason we have hitherto investigated the action of strychnine only in doses somewhat higher than the ordinary therapeutic doses, and have obtained a very marked increase of the rate of epinephrin output, both in the dog and cat, up to ten times the initial output. Asphyxia was excluded by artificial respiration. The effect of strychnine is not transient and may outlast for a considerable time the convulsant action, when such is present. The maximum increase is not necessarily found in specimens of adrenal blood taken immediately after the appearance of the symptoms of strychnine action on the cord. Thus, in one experi-

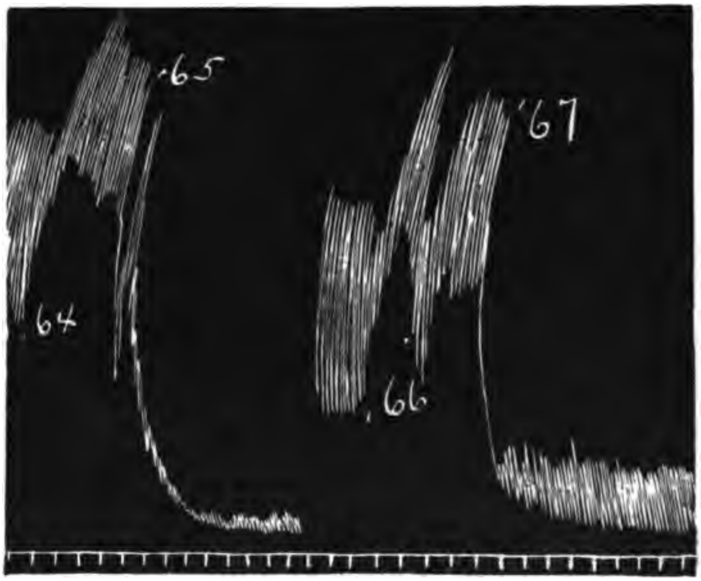

Fig. 16. Intestine tracings. Bloods from cat 4. At *64* and *66* Ringer was replaced by jugular blood and this was replaced at *65* by the 8th adrenal blood specimen and at *67* by jugular blood to which was added adrenalin to make a concentration of 1:1,000,000. All the bloods were diluted with 10 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

ment the output of epinephrin in blood collected immediately after the onset of the symptoms was shown to be three times the initial output, whereas one hour later it was four times the initial output. In another experiment the output was doubled with the onset of the strychnine symptoms but more than an hour later, when the symptoms had apparently completely subsided, the output was three times the initial output. In a third experiment, on a deeply anesthetized animal in which the dose of strychnine employed caused no convulsions, the epinephrin output was definitely increased in a blood specimen taken a few minutes after the injection of the strychnine, but the increase was twice as great in a specimen collected a little more than an hour thereafter. It is not always the case, however, that the later specimens show a greater increase than those collected soon after the administration of strychnine. In one experiment, for example, the output soon after the development of the strychnine symptoms was ten times the initial output, while a specimen taken three-quarters of an hour later showed six or seven times the initial output. Of course, the extent of the increase varies in different experiments. The subject is being investigated further, especially the action of smaller doses, and the details will be published in a subsequent paper. The possibility that the stimulating effect of strychnine upon the epinephrin output may enter as a factor into some of the pharmacological and therapeutic actions of the drug is obvious, but we have at present no evidence on this point.

SUMMARY

The rate of output of epinephrin in dogs and cats, after the blood pressure had been permanently lowered by exposure and manipulation of the intestines, by partial occlusion of the inferior vena cava, by hemorrhage and by "peptone" injection, was found to be the same as before the lowering of the blood pressure, within the limits of error of the methods used for assaying the epinephrin.

A marked increase in the rate of output of epinephrin was produced by strychnine.

BIBLIOGRAPHY

(1) Stewart and Rogoff: This Journal, 1917, xliv, 149; Journ. Pharm. Exper. Therap., 1917, x, 1.
(2) Popielski: Pflüger's Arch., 1918, clxx, 245.
(3) Burton-Opitz and Edwards: This Journal, 1917, xliii, 408.

(4) Stewart: Journ. Exper. Med., 1912, xv, 555.
(5) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1916, viii, 479.
(6) Bedford: This Journal, 1917, xliii, 235.
(7) Stewart, Rogoff and Gibson: Journ. Pharm. Exper. Therap., 1916, viii, 205.
(8) Stewart: Journ. Exper. Med., 1911, xiv, 377.
(9) Stewart and Rogoff: Journ. Exper. Med., 1917, xxvi, 613.

ON THE ELECTRICAL RESPONSES OF THE EYE TO STIMULATION BY LIGHT OF VARIOUS WAVE-LENGTHS

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HISTORICAL INTRODUCTION

Holmgren (1) is accredited with the discovery that when light is allowed to fall upon the eye of a frog which has been kept in the dark and again when the light is removed, there is an increase in the positive direction of the current present during darkness. He found that the strength of these retinal currents is, within limits, proportional to the intensity of the light; also, that the onset and removal of light is attended with the same electrical changes when the posterior half of the eyeball only is illuminated.

Dewar and M'Kendrick (2), who independently of Holmgren and at about the same time made investigations upon these electrical changes caused in retinae by light, proved experimentally, *a*, that the impact of light on the eyes of members of the following groups of animals, viz., mammalia, aves, reptilia, amphibia, pisces and crustacea, produces a variation amounting to from 3 to 10 per cent of the normal electromotive force existing between the corneal surface and the transverse section of the nerve; *b*, that this electrical alteration may be traced into the brain; *c*, that those rays which we regard as most luminous produce the largest variation; *d*, that the alteration of the electrical effect with varying luminous intensity seems to follow very closely ratios given by the psycho-physical law of Fechner; *e*, that the electrical alteration is due to the action of light on the retinal structure itself, as it is independent of the anterior portion of the eye, eliminating, therefore, the natural supposition that the contraction of the iris might produce such a result; and *f*, that it is possible by experiment to discover the physical expression of what is usually called in physiological language, fatigue.

Kühne and Steiner (3) investigated chiefly the reactions of the isolated retina. They found that the electrical change on lighting and darkening is a complex one. The reaction is divisible, they say, into two parts: the first one, due to the onset and continuance of illumination, consists of a negative variation preceded by a positive; and the second, caused by the disappearance of the light, consists of a simple positive variation. No current was obtained from the posterior half of the eyeball from which the retina had been removed, the pigment layer being left behind. Hence the pigment epithelium possesses no electromotive power.

Waller (4), whose brochure on *The Signs of Life* is most fascinating, concludes from his experimentation: *a*, A fresh normal eyeball manifests a positive current which gradually declines to zero and becomes reversed. By *positive current* Waller refers to the current of response due to the action of light on the retina which is from the nerve—that which is not directly stimulated by light—to the retina; that is, from the less excited to the more excited. *b*, On exposure to light the normal current, whether positive or negative, undergoes a positive variation. *c*, The magnitude of the response to light increases with the duration of illumination. *d*, The magnitude of the response to light increases with the strength of illumination. *e*, Colored lights act in the same direction and in accordance with their luminosity.

Gotch (5) made use of a capillary electrometer which recorded the rapid alterations of current present in the retinal responses. He divided the electrical reactions of the eye to light into three portions: *a*, the rise due to sudden illumination or the "on-effect;" *b*, the continuous change occurring during the continuance of the illumination, and *c*, a second rise due to the sudden change from light to darkness or the "off-effect." The results seem to indicate the localization of two photochemical substances in the posterior half of the eyeball, these being a substance reacting to light and a substance reacting to darkness. Each reaction is of the same type.

Einthoven and Jolly (6) used a string galvanometer and an arc as a source of illumination with suitable diaphragms. They conclude: *a*, The photo-electric reaction of an isolated eye is specially adapted to the study of the effect of stimuli of very different intensities. In their experiments light stimuli were used whose energy varied from 3.05×10^{-12} to 3.95×10^{-2} g. cal. per second. *b*, The form under which the photo-electric reaction manifests itself under different conditions gives ground for the supposition that there occur in the eye three separate processes and that each of these may be dependent upon a separate substance. *c*, The first substance reacts more rapidly than the other two. On lighting it develops a negative, on darkening, a positive potential difference. *d*, The second substance reacts less rapidly than the first and in an opposite direction or sense. On lighting it develops a positive, on darkening, a negative potential difference. *e*, The third substance reacts in the same sense as the second but much more slowly. *f*, For each of the three substances the rule holds good that with moderate and strong light the energy of the stimulus increases much more rapidly than the energy of the reaction.

Other important researches, in a general way confirmatory of certain of the conclusions previously arrived at, have been made by Fuchs (7), DeHaas (8), Piper (9), Ishihara (10), Bruecke and Garten (11).

RÉSUMÉ OF EXPERIMENTATION AND OPINIONS UPON THE EFFECTS OF DIFFERENT WAVE-LENGTHS AND THE QUESTION OF COMPLEMENTARY OR ANTAGONISTIC INFLUENCES

Since we are particularly interested in this research in the investigation of the effects of various colors or wave-lengths of light upon the retinal response and in the question of corresponding or antagonistic influences, such as would be demanded by the Hering theory, for example, it is pertinent to present briefly the experimental evidence obtained up to date upon these points.

Gotch (12) exposed an excised frog's eyeball which had been kept in the dark to the red end of the spectrum for 1.6 seconds: the response commenced after a delay of 0.225 second and reached a maximum of 0.00045 volt at 0.8 second. After a period of five minutes' rest the result was checked. During a further period of five minutes' wait the position of the chamber carrying the eye was shifted to the green region of the spectrum, to which the eye was exposed for 2.7 seconds. The response began after a delay of 0.18 second and reached a maximum of 0.0004 volt at 0.6 second. And so on for the violet end, the delay in response being 0.22 second and the maximum response 0.0003 volt at 0.6 second. In conclusion Gotch says that the maximum response to red light was 0.0004 volt, green light 0.0005 volt and violet light 0.00024 volt. By means of a slit the "amount of light passing through the grating used could be always adjusted until it corresponded in photometric value with that of the candle." In concluding his remarks upon these investigations he says (13):

> If it is permitted to associate these results with the well-known theories of colour vision, then it is evident that the Young-Helmholtz theory which assumes three primary colour reacting substances, red, green, violet, is in remarkable accordance with the data just put forward. In one respect only are the photo-electric responses not in accord with that theory, for although the responses appear to show that there is a substance capable of being stimulated by darkness there is no fourth reacting substance postulated in the theory. On the other hand, this is the only fact which is in any sense applicable to the rival theory advanced by Hering and even this is not in accordance with the assumption of Hering that the sensation of black, although caused by stimulation, is brought about by a change of opposite chemical type to that produced by white. The photo-electric responses, being all of the same fundamental type, are not in harmony with the assumption that there are three reacting substances, a red-green one, a blue-yellow one and a white-black one, and that the process is of opposite sign (association or dissociation) in each member, a process of one sign occurring with red, blue or white, whilst that of the opposite sign occurs with green, yellow or black.

In his first paper Gotch (14) concludes that the results appear to indicate the localization of two photo-chemical substances in the posterior half of the eyeball, these being a substance reacting to light and a substance reacting to darkness. Each reaction, he says, is a change of the same type, but for the change to occur the eye must be previously adapted, *i.e.*, the substances must undergo phases of metabolism under conditions opposite to those which evoke the reaction effects.

Waller (15) writes:

> We may see whether or no complementary colours have opposite electrical effects, and whether the excessive action of a given colour favours or disfavours the action of its complementary. . . . . The effects of all sorts of colours were of the same sign. The joint effect of two complementary colours was practically the sum of their separate effects. And the prolonged excessive action of a given colour fatigued the retina to that colour just as much or as little as to the complementary colour. All colours, in fact, as regards the electrical response they elicit from the retina, give that response of the same sign and seem to act in the same direction, more or less powerfully according as they are more or less luminous.

Thus, *e.g.*, in a given series of trials, the responses had the following relative magnitudes:

Red	4
Yellow	16
(White)	33
Green	12
Blue	9

Furthermore, all the experimental facts obtained—viz., the transitional responses from positive to negative, the immediate conversion from positive to negative by compression, the initial period of hesitation—point to the conclusion that the retina, when exposed to light, is the seat of two contrary electromotive changes. And Waller says,

> It matters little whether you imagine two single processes or one double process behind the movements of the machinery. . . . . We are all acquainted with Hering's theory of colour-vision setting forth that contrary processes of dissimilation and assimilation are aroused by complementary colours. . . . And whether or no we happen to believe that, e.g., a red light is katabolic and a green light anabolic, we assuredly do believe in what has been termed by Bernard the axiom of general physiology—that katabolic analysis and anabolic synthesis are inconceivable apart from each other. I think that we should not hastily admit that our double electrical change is presenting us to another aspect of a familiar if somewhat nebulous colour-theory: I think we have no ground for assuming that our conclusions mean anything more than the old axiom behind a new face. A process necessitates the anti-process, and if the process is attended by an electrical change of given sign, we may expect that the anti-process will entail an electrical change of opposite sign. The chemical changes taking place in living matter are in general reversible changes.

PURPOSES OF THE PRESENT EXPERIMENTATION

We are presenting such experimental evidence as we have been able to obtain, after a number of repetitions, which bears upon:

1. The directions of the electromotive forces,—i.e., whether the current is from the nerve to the retina or vice versa,—under exposures to varying wave-lengths of light.
2. The question as to whether or not the excessive action of a given monochromatic radiation will promote the subsequent action of its complementary.
3. The general physiological problem of dissimilation and assimilation, or katabolism and anabolism.
4. The possible bearing of the experimental results obtained upon the theories of color vision.

We shall not attempt in this paper any theoretical discussions as to the possible physical, chemical or physiologic explanations of the effects observed, since we are of the opinion that too few facts are at hand to warrant the addition of another guess to the many that already exist.

APPARATUS

The schematic arrangement of the apparatus which was used is shown in figure 1.

Optical apparatus. The source of light, *S*, was a carbon arc, hand feed, supplied by an alternating current dynamo, *D*. The quartz lens system L_1 threw the light upon a slit and thence to a lens L_2 and a prism *P* carrying on one face a grating replica. A continuous spectrum was formed upon a cardboard screen marked into wave-lengths and represented by *R Y G B* in figure 1. This whole optical arrangement is conveniently assembled in the spectrum projector manufactured by the Bausch & Lomb Company. One of these devices was used through this work.

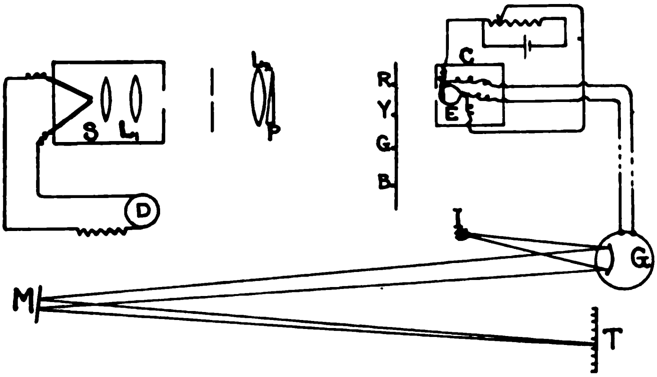

Fig. 1. Ensemble of experimental apparatus

The eyeball, enclosing case, etc. All eyes used were freshly excised from young dogs in healthy condition. In no case was experimentation with an eye started later than two hours after enucleation. The eyeball was allowed to rest on a hollowed-out pad of soft wax and was held in position by means of an arm swinging from a rod running the length of the enclosing case. To this same rod were fastened the non-polarizable electrodes. Every precaution was taken to avoid mechanical pressure or injury to the eyeball.

The front of the containing case, *C*, was perforated by a small aperture carrying a swinging cover to admit or exclude light.

The electrodes made contact respectively with the cornea and with the nerve stump.

Galvanometer. The galvanometer was of the Thomson type and had a sensitiveness of 1.2 x 10^{-9} ampere, i.e., one millimeter deflection at one meter corresponded to a current of 1.2 x 10^{-9} ampere. Such a sensitiveness was not sufficiently high for the purposes desired judging by previous experimentation (15, p. 151). We therefore increased the sensitiveness about tenfold by using a concave mirror on the galvanometer and so placing a luminous source, *I*, as to cause a beam of light to be reflected from the galvanometer mirror across the room to a plane mirror, to be finally focussed on a transparent screen at *T* (fig. 1). This method enabled us to measure an indicated electromotive force of 0.00016 volt for one millimeter scale deflection.

Compensating potentiometer. When an excised eyeball is connected up through non-polarizable electrodes touching the cornea and nerve, a current of injury is found which must be compensated or eliminated. This can be accomplished as diagrammed in figure 2. A constant voltage cell may be short-circuited through a high resistance, *R*, from which any desired potential difference may be tapped off and applied across the eyeball in such a direction (by proper connection of the two terminal points of the battery to the eyeball) as to bring the galvanometer reading back to its initial zero point. In this manner, provided the compensating electromotive force demanded does not vary with time, the effects obtained by illumination are genuine and are not complicated by current or injury effects.

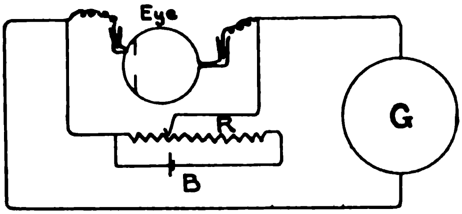

Fig. 2. Detailed diagram of compensating potential device.

Exposure to various parts of the spectrum. The case containing the eyeball was not disturbed during an evening's experimentation. The spectrum projection apparatus, which was fastened to a single supporting base, was turned so as to allow the eye to be exposed to any desired portion of the spectrum.

Electrical screening. The case holding the eye and the electrical portions of the spectrum projection apparatus were suitably screened with wire cages connected to earth to prevent spurious effects upon the galvanometer leads, etc. The leads, in turn, were wound non-inductively and reasonably well protected from stray electrical effects.

METHOD OF INVESTIGATION

a. Differences in present methods as compared with those previously pursued. In a general way, previous experimenters have exposed excised eyes (usually frogs' eyes) to luminous energy for periods of time varying from a few seconds to a minute and then to equal or longer periods of darkness and have thereby investigated the nature of the response and recovery curves. They have paid little or no attention to the general rise or fall of potential after stated periods of exposure. Hence we have, in general, exposed an excised eye to a certain portion of the spectrum for from four to five minutes and then have waited, the eye being screened, for an equal or longer period of time, before again exposing to a different portion of the spectrum. The general method of procedure has been to investigate the *end changes* in potential difference after alternate exposures to long (e.g., red) and short (e.g., blue) visual radiant energy. In our investigations on the effects of exposure to a color complementary to that used immediately preceding, we have made our experiments after the eye had been in use for some time and had ceased to show appreciable variations in response, hence may be conceived to have been fatigued to a given color. In the experiments of Waller we are led to believe that the responses to red, green, etc., were taken rather rapidly one after the other.

b. Sources of error. 1. In all such experimentation it is necessary to apply across the cornea and optic nerve trunk a compensating electromotive force to annul the current of injury. If is, of course, possible that this current decays with time or reverses its direction. To obviate this trouble we waited some little time before commencing our observations. Repeated series of observations with different eyes, the order of exposure to different radiations being considerably changed, aided in the eradication of false conclusions due to such an error.

2. The fact that the electrodes may be polarizable, whereas they should be absolutely non-polarizable. The usual form of clay boots with zinc and zinc sulphate were used. These are presumably the best known.

3. The effects due to mechanical pressure and jar. It has been found that mechanical pressure, etc., will modify or reverse the character of responses. Extreme care was exercised to prevent such pressures or jars.

4. Condition of the eye. The normal, fresh, uninjured eyeball gives entirely different types of response as compared with the partially injured or moribund eye.

5. The periodic variations of the sensitive galvanometer used. A type of galvanometer, such as the Thomson, when highly sensitive is subject to shifts of the zero in a rather rhythmic fashion. Naturally the repeated sets of observations, referred to above, aided in the elimination of this source of error. We refer to this source of error in particular, since some of the curves presented in this paper might be accounted for in their forms by this rhythmic variation of galvanometer swing were it not for the fact that several sets of observations showed that no such result was possible.

6. Fluctuations in the arc used.

All these sources of error would, however, be likely to be present or absent in greater or lesser degree in each different eye tested on different days and hence cannot vitiate the general results of the experiments to be described below.

RESULTS

a. The form of the photo-electric reactions to the initial light stimuli of different wave-lengths. A freshly excised dog eye was taken and, after preliminary arrangements, was initially exposed to a region of the spectrum such that λ5900 (sodium line) fell upon the center of the pupil. The total time of exposure was four minutes. The plotted results are shown in figure 3; in this figure the ordinates represent electromotive forces and the abscissas the time in seconds. The maximum value of the response to yellow light taken at any point on this curve, as judged with respect to its own minimum, is practically 20 divisions or + 0.0032 volt. The reason for the recurrent maxima, as *A*, *B*, *C*, etc., in figure 3, will be discussed later. The difference between the initial electromotive force (just before exposure, which was zero) and the final zero or reading after four minutes is + 10 divisions or + 0.0016 volt. The eye was then screened from the radiation but the process apparently continued such that in the end the final reading after six minutes of darkness was 30 divisions above the initial zero or +0.0048 volt. The lines drawn through the points *A*, *B*, *C*, etc., and the minimum points *D*, *E*, *H*, etc., are parallel and show the gradual *rise*, from the fiducial or arbitrary balance point, of the potential difference under exposure to the yellow (sodium light) region of the spectrum. This would indicate the constant growth of a positive electromotive force independent of the superposed maximum responses. It will also be noticed that in no instance is there a return to the initial zero, nor again any fluctuation indicating a reversal of sign in current or potential difference: this would be indicated on the plot by experimental values below the original zero point.

In contradistinction to these results obtained with λ5900, figure 4 shows the form of responses and potential differences when another freshly excised eye was initially exposed for four and a half minutes to the violet λ4310. The color represented by this wave-length is roughly complementary to that of λ5900, the effects of which have been described in conjunction with figure 3. The maximum value of the electrical response to λ4310 (at center of pupil) taken at any point during the first two or three minutes as judged with respect to its own minimum is practically −15 divisions or −0.0024 volt. The difference between the initial electromotive force—which was zero under the compensating potential device—and the final reading after four minutes' exposure was practically −15 divisions or −0.0024 volt. The eye was then screened from radiation and the end reading (after six minutes' rest) obtained, indicating a total difference between the initial and the end reading (over the whole period of time) of −12 divisions or −0.0019 volt. This is less than the general indicated difference, up to three minutes' exposure, of −0.0024 volt. However,

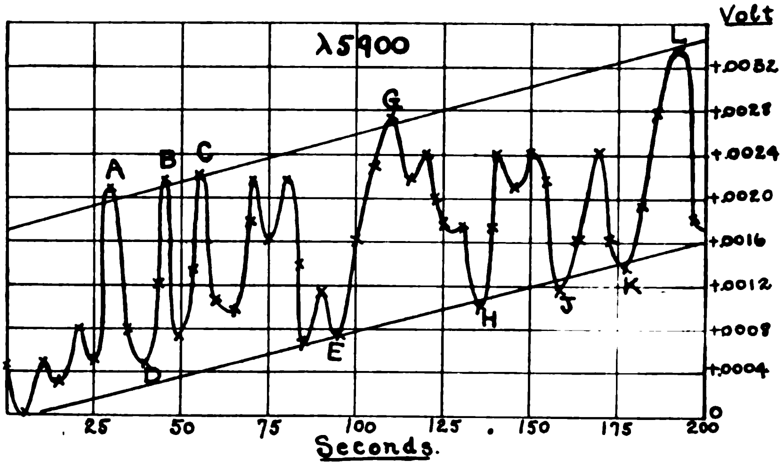

Fig. 3. Potential-time curve due to stimulation by λ5900

the concluding portion (from abscissa 180 to 270 seconds) shows a tendency for the contrary or opposite electrical effect (?) to exert itself more pronouncedly. The lines drawn through the points *A*, *B*, *C* and the maximum, electrically considered, points *D*, *E*, and *F* are parallel and show a gradual *fall* from the arbitrary balance starting-point under exposure to λ4300. This would indicate the growth of a *negative* electromotive force independent of the superinduced fluctuations giving rise to maxima and minima. It is also to be noted that there is, with one or two exceptions, no return to the initial zero nor any indications that there is a reversal of sign of potential difference or current except in the latter part of figure 4.

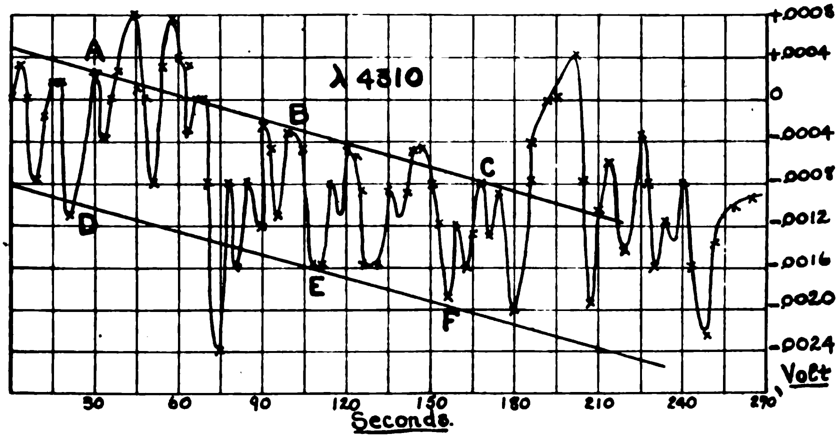

Fig. 4. Potential-time curve due to stimulation by λ4310

By comparison therefore we find that λ5890 produced *positive* potential differences and positive currents as well as a *gradual rise* in the zero, i.e., the positions of the minima in the various fluctuations observed, in a positive direction: λ4310, roughly the complement of λ5890, produced *negative* electromotive forces and a *gradual decline* (positively considered) or gradual increase (negatively considered) in the position of the arbitrary zero point in a negative direction. In the case of exposure to λ5890 the fluctuations of potential difference are always in such a direction that the maxima indicate *increased positive potentials at the nerve*, with returns to minima which are still always positively above the fiducial zero. In the case of exposure to λ4310, however, the fluctuations of potential difference are always in a direction indicative of *increased positive potentials at the retina*, hence *reversed* potentials as compared with the first named set of conditions.

b. The forms of the retinal responses produced by seriatim exposure to light stimuli of different wave-lengths. Figures 3 and 4 give initial effects in two different eyes. In figure 5 have been plotted seriatim the results obtained by exposure of the same eye to λ5900, first, for a period of four minutes, a period of six minutes' rest, and a second exposure to the blue-violet λ4860, the complementary color to λ5900, for a period of four minutes. The same general conclusions are to be drawn from the seriatim exposures as from the individual exposures in separate eyes. In the case of exposure to λ5890 there is a maximum rise of electromotive force in a positive direction of 0.003 volt: the difference between maxima and minima in the fluctuations of potential is 0.0024 volt (practically): there is a steady rise in the permanent electromotive force during the continuance of the illumination amounting to about 0.0012 volt. After the six minutes' rest, the initial exposure to λ4860 produced but slight, although decreasing, potential effects during the first 25 seconds, to be immediately followed by an increased electromotive force in an opposing direction such as to carry back the positive potential established under illumination by λ5890 toward the initial zero. The conflict of the two processes is very clearly indicated in the record of the responses to λ4860. The curve as a whole shows the establishment of a contrary electromotive force as compared with that established by λ5890; a decided reaction to λ4860 at the end of 25 seconds' exposure and the gradual decrease of the positive potential difference established under stimulation by λ5890. Furthermore,

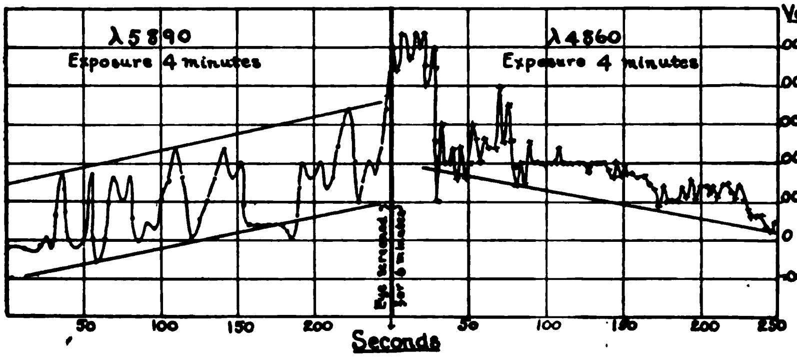

Fig. 5. Potential-time curve due to stimulation by λ5890 for four minutes followed by stimulation by λ4860 for four minutes.

both these curves, as well as others obtained by us (also see curve by Waller, *Signs of Life*, p. 26) show the first *pronounced* effect to light stimuli after 25 to 35 seconds of exposure.

Figures 6 and 7 present in graphical form the experimental results obtained when an eye was exposed to luminous stimuli of various wave-lengths seriatim. The manner of procedure in obtaining these curves was as follows: A fresh eye, an hour after excision, was connected into the galvanometer circuit and the galvanometer needle brought back to its original zero through the agency of the compensating potential device. The eye was then exposed to a certain region of spectral energy for four minutes after which the eye was again stimulated by luminous energy of entirely different wave-length. This process was continued over several such steps in the case of each eye investigated. The initial stimulation was obtained from entirely different spectral regions in the four curves presented in figures 6 and 7. These curves are plotted with the abscissae indicating the region of light stimulation:

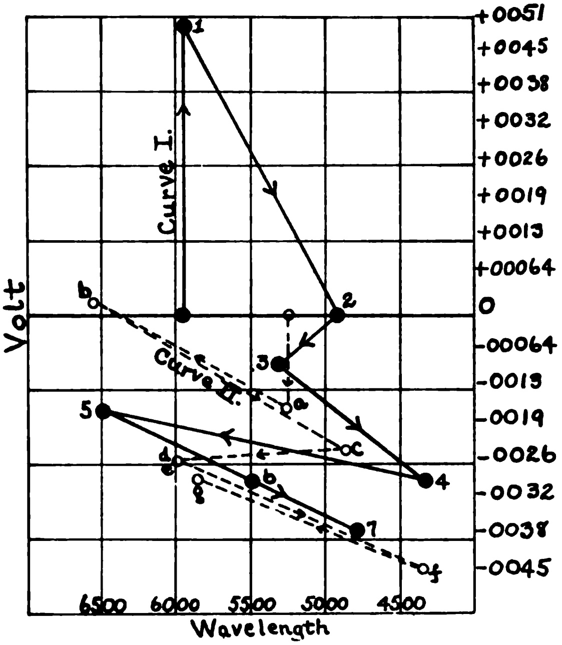

Fig. 6. Summarized results of the potential effects of seriatim exposures to various wave-lengths.

the ordinates represent the change in potential as registered by the galvanometer at the end of each cycle, namely the four minutes of exposure and the six minutes of rest. The order of stimulation, and regions used therefor, are indicated by the serial numbers *1*, *2*, etc., or by letters *a*, *b*, *c*. In curve *I*, figure 6, for example, the eye was stimulated by light of average (i.e., striking the center of the pupil) wave-length λ5890 (sodium yellow). The potential after four minutes' exposure and six minutes' rest had risen to 0.0049 volt as indicated at the point marked *1*. The eye was then stimulated by λ4860 for the same length of time and occluded, when the potential was found to have dropped down from 0.0048 to zero. Exposure to λ5300 reversed the potential and gave −0.007 volt; λ4350 carried the final reading to −0.0028 volt; stimulation by λ6500 reduced the potential to −0.0016 volt or carried it back toward the initial zero point; λ5500 increased the negative potential and λ4800 carried it to −0.0038 volt, at which point the experimentation was discontinued. In curve *II* of this figure, the initial stimulus was furnished by λ5250 (green) and ultimately gave a *negative* potential increase of −0.0016 volt indicated by the point *a*. Exposure to λ6563 (red) *reversed* the potential and gave a final value of 0.00032 volt; λ4860 *reversed* it and carried it back to −0.0024 volt and so on. The points marked *d* and *e* were dual

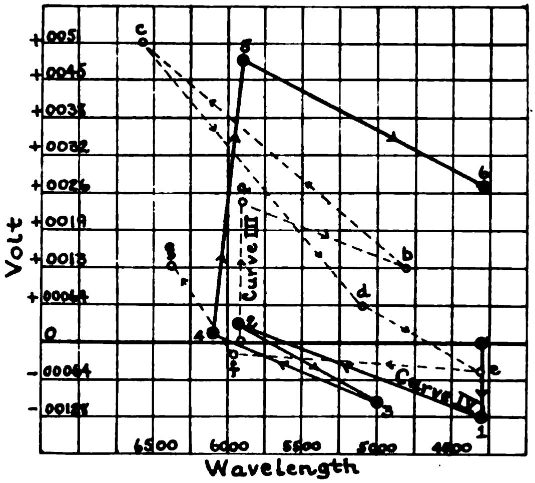

Fig. 7. Summarized results of the potential effects of seriatim exposures to various wave-lengths.

exposures to λ6000 and indicate that the second exposure to the same wave-length produced no further changes in potential. In fact the galvanometer needle remained practically quiescent during the whole of the second stimulation by λ6000.

It is of interest to note that in curve *I* λ4860 (point *2*) is practically the complement of λ5900 (point *1*) and in curve *II* λ5250 (point *a*), the complement of λ6563 (point *b*), and that in each of these cases the potential effects due to one are practically reversed and annulled by those due to the second member mentioned. The maximum potential effects were +0.0049 volt for λ5890 and −0.0016 volt for λ5250.

Curves *III* and *IV* of figure 7 are self-explanatory in view of the discussion in connection with figure 6.

We conclude, therefore, from these and other similar sets of curves obtained, that there is evidence of two processes operating in the production of retinal electrical potentials and that, in general, the shorter wave-lengths of light produce negative potentials (from retina to nerve) while the longer wave-lengths produce positive potentials (from nerve to retina).

c. *Complementary colors and reactions.* We have in two instances investigated the effects of luminous energy of a wave-length approximately complementary to the spectral region last used to stimulate the eye, under the condition that the eye had been under experimental use for some time and reached the stage when the responses to stimulation were of a minor character and did not show the pronounced and rapid changes of potential indicated in the initial stages of experimentation—such, for example, as diagrammed in figures 3 and 4. The history of the eye from which figure 8 was obtained is as follows: *a*, initial stimulation λ5900; *b*, λ4860; *c*, λ6563; *d*, λ5000; *e*, λ4300, involving a total time of 45 minutes. Immediately previous to the obtaining of this curve the eye had been exposed to λ4300 for four minutes. After a period of six minutes in darkness it was exposed to λ5890 (sodium light) for two and a half minutes. Upon "ironing out" the irregularities, the potential-time curve has the form of the heavy line *A-B-C-D-E*. At the point *E* (after 2.5 minutes) the eye was stimulated with λ5370 (green) and the general rise of the potential positively (i.e., from nerve to retina) is indicated by the portion of the curve *E F*, with a maximum electromotive force of +0.0020 volt. The results of this experimentation indicate quite clearly that λ5370 is more nearly the complement of λ4300 than is λ5890 and that λ5370 produced a decided change in potential and in the *reverse* direction as compared with the immediately preceding stimulant, λ4300.

Figure 9 gives a set of conditions somewhat the opposite of those shown in figure 8. After five exposures to various portions of spectral illumination in the manner detailed in connection with the discussion of figure 8, the eyeball was exposed for four minutes to λ5890. The further excitation under λ4300 produced but slight effect whereas, when the pupil was illuminated with λ4860, the increase of *negative* potential (i.e., from retina to nerve) is decisively indicated in the plotted results. Hence λ4860, the complementary color to λ5890, produced a decided change in potential and in the reverse direction as compared with the immediately preceding stimulant.

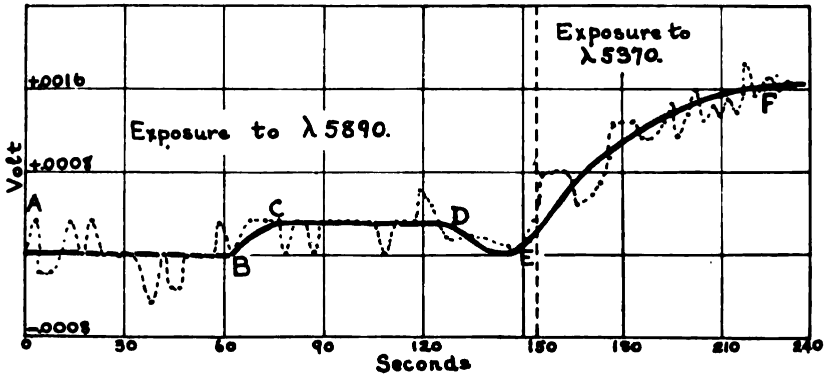

Fig. 8. Potential effects due to previous stimulation by λ4300 followed by stimulation with λ5890 and λ5370.

These facts are further substantiated in a general way by the results shown in figure 5, in which a stimulation by λ5890 is immediately followed by its complementary color, λ4860.

d. Possible explanations of the pronounced maxima and minima (or fluctuations) in the potential-time relations occurring during the early stages of luminous excitation. 1. Rhythmic variations of sensitive galvanometer system. Attention has been called to this under the heading "sources of error." Several sets of observations upon the "wandering of the zero" of the galvanometer needle were made on different days and evidenced much smaller values and entirely different periods as compared to the effects obtained with the eye under stimulation. Furthermore, a comparison of the results obtained in the initial stages of experimentation, e.g., figure 3, with those obtained during experimentation with the same eye about an hour later, e.g., figure 8, show that galvanometer periodicity is not an appreciable factor and cannot account for the results obtained.

2. Mixed responses characteristic of transitional changes. Waller (15) has classified the responses to light as falling into three groups, the second of which is known as the *transitional* type; that is to say, transitional between the normal and the compressed or partially injured eye. This transitional type of response gives, according to Waller, potential-time responses which bear some similarity to those under discussion. It is possible, of course, that the experimental device used by us may have caused pressure, but extreme care was exercised to see that the eye rested easily on its support and the rods carrying the electrodes were so arranged as to permit of contact without any pressure. The eyes were always fresh, hence moribund conditions and changes can be cast out. Very suggestively Waller points out the composite effect of the superposition of two opposite and presumably synchronous electromotive forces. In nearly all the curves obtained by us we found that the first appreciable change in potential occurred after about thirty seconds' exposure and that during the first ten to twenty seconds there was some hesitancy in response. An inspection of Waller's curves, for example, shows that the maximum potential was attained in about half a minute. Previous experimenters do not appear, however, to have exposed the eye continuously for several minutes to stimulation but have preferred to alternately expose

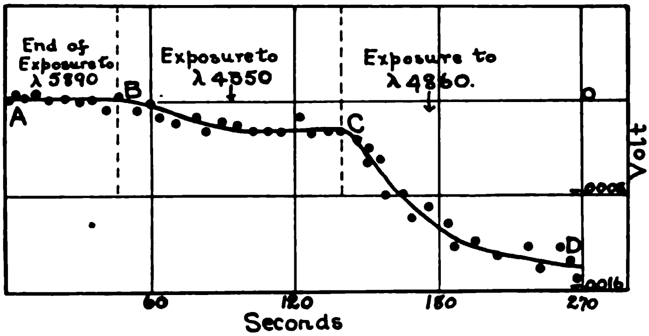

Fig. 9. Potential effects due to previous stimulation by λ5890 followed by its complementary, λ4860.

and then rest the eye; hence a comparison of results in this particular is not wholly specious.

3. Fatigue. That which is to be presented in this paragraph may be but a process of reasoning from analogous visual effects, such as after-images, which may depend for their explanation upon the material presented under the succeeding subhead. If there are two separate and opposite sources of electromotive force, as seems reasonable from all the data obtainable, then continuous exposure may produce a cessation of or fatigue in one of the processes, thereby giving the opposite process the upper hand. Direct exposure of a living eye to illumination will, when carried out in such a manner as to prevent a wandering of the fixation, produce effects analogous to those fluctuations which we have obtained. By fixedly observing a brilliant light source, periods of cessation of luminous sensation will occur followed in a few seconds by a sensation of luminosity. Likewise the phenomena of positive after-images evidence the fact that there are marked variations in the colors, etc., of the images seen. For, if the filament of an incandescent lamp is viewed for a few seconds and the eyelids then closed, a positive image of great distinctness will be seen which will persist for some time. If the eyes are kept closed the less illuminated portions of the image disappear, while the more illuminated parts change color, becoming bluish, etc., in turn. The image finally disappears only to reappear with a repetition of the foregoing series of color changes: the image finally reaches a state of decay when it appears darker than the surroundings. Negative after-images, which possess the complementary colors to the original stimulus, may be thought of as fatigue or, again, as a metabolic change of opposite character to that produced by the original stimulus. The excised eyes used in our experimentation, being fixed and hence suffering retinal irritation and stimulation at one and the same region continuously, may reasonably be supposed to have undergone processes of fatigue and hence have exhibited "re-bounds" similar to positive after-image effects.

4. Polarization effects. The action of luminous stimulation upon the retina must lie without doubt in the ionization processes set up in the visual receptor cells. What these processes are or what the nature of the changes produced is still an enigma. However, ionization involves a dissociation into positively and negatively charged atoms and electrons. A stimulation by light must, therefore, set up a certain process of ionization whereby the positive ions may be thought of as moving in general in one direction and the negative atoms (if such exist) and electrons as moving in opposite directions. Photo-electric activities of metals, such as sodium, show a discharge of negative electrons under the action of light, the rate of discharge depending upon the metal and the character of the illumination. However, we need not think of motion of both sets of ions but of one only, for example the electrons, and that membranes and tissues are more permeable to one than to the other of the ionized products. We may suppose that the retinal rods and cones contain certain electrolytes or ionizable substances which, in the absence of light, are ionized to a degree dependent upon the equilibrium constant of the reversible dissociation action. The introduction of light into the system alters the value of this constant by increasing the rate of the dissociating change without affecting that of re-association. It seems not improbable, therefore, that the passage of one set of charged particles out of the receptor cells along the nerve trunks will thereby increase the potential difference between retina and nerve and cause a building up of a surplus of the second kind or set of charged products of the ionization process which, by accumulation, will inhibit and in large measure sweep out the oppositely charged products of dissociation when the concentration becomes sufficient

We know that when one end of a wire is placed in contact with the cut end of a nerve and the other is touched to the ininjured nerve sheath, a current flows from the sheath to the cut portion. Hence the former is positive with respect to the latter. The Nernst-Lillie theory explains this by supposing the nerve tissues to contain an ionizable substance (or substances) the positive components of which diffuse through the sheath more readily than the negative. The sheath has, therefore, a differential permeability with respect to ions. When the cut end of the optic nerve is connected by a wire to the retina we find the retina to be more negative than the nerve end under certain conditions and *vice versa* under other conditions of stimulation. We must, therefore, it seems to us, postulate the existence of at least two ionizable substances in the receptor cells. One of these substances must give maximum ionization effects under yellow, for example, and the other similar effects under blue-violet stimulation. For one of the substances the negative ions of the receptor cells diffuse more readily than do the positive ones, while the reverse is true for the second substance.

Or, again, it is possible to look at the results from a different point of view, assuming only the opposing action of potentials set up at the receptor cells and the nerve fibers. L. T. Troland (16) in writing on the visual receptor process says:

> It is evident that if, in point of fact, the polarization of the receptor cells is opposite to that of the nerve fibres, there must be a constant tendency for these two tissue elements to depolarize each other, at their junction. In the case of the nerve, such depolarization will result in an impulse, which will pass along the fibre to the brain centers; in the case of the receptor cells, it will result in a momentary increase of permeability and the diffusion of a certain amount of substance through the cell membrane into the surrounding medium. After any single interaction of this sort, both the nerve fibre and the receptor cell will begin to recover their differential permeability as soon as the depolarization has reached its maximum. . . . . The reëstablished differential permeability and polarization of the nerve fibre consequently is maintained until the differential permeability of the receptor cells reaches, in the course of its recovery, a point at which the resulting receptor polarization is again able to neutralize that of the nerve fibre, and thus to cause a second depolarization of the latter. This intermittent action will obviously continue *ad infinitum*, if the electrolytes of the tissues are kept at the requisite concentrations.

POSSIBLE BEARING OF THIS EXPERIMENTATION UPON HERING'S THEORY OF COLOR PERCEPTION

The Hering theory assumes the existence in the retina of three visual substances, a whitish-black, a red-green and a yellow-blue substance. In each pair one color is complementary to the other. The theory also assumes that the different colors affect the different substances differently. Red stimulation induces a katabolic (dissimilirung) in the red-green substance, giving rise to a sensation of red, while green light gives rise to a constructive or anabolic change (assimilirung) in the same manner, producing the sensation of green. In the same way katabolic changes in the yellow-blue substance induced by yellow light give rise to the sensation of yellow, while anabolic changes are induced by the shorter visual wave-lengths of light. These theoretical considerations of anabolism and katabolism find their experimental counterparts in the positive and negative potential effects produced by various wave-lengths of light as outlined in the preceding paragraphs. These experiments point strongly to the conclusion that there are two distinct and opposing processes which may be designated as assimilation and dissimilation.

In figure 10 we have reproduced a diagram from Foster's *Text-Book of Physiology* to illustrate the Hering theory of color vision. The line *r g*, which encloses a space shaded vertically, is intended to represent the effects of rays of different wave-length on the red-green visual substance. In the red, orange and yellow, up to λ5800, the effect is katabolic: beyond λ5800 the effect is anabolic or one of assimilation. Similarly, the line *y b* represents the behavior of the yellow-blue substance and is shaded horizontally in figure 10.

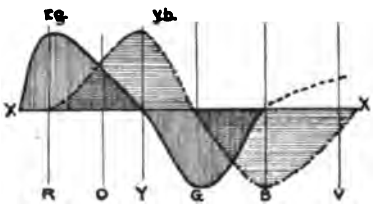

Fig. 10. Schema illustrative of Hering's color theory

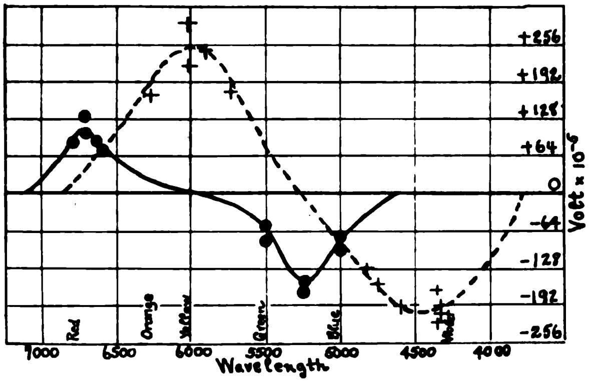

Fig. 11. End potential effects plotted against wave-lengths of light showing the similarity of potential curves to those demanded by the Hering theory.

Figure 11 gives a résumé of the retino-nerve potential differences due to various light stimuli obtained from various sets of observations made by us. A few data have been cast out in the plotting of these curves, otherwise the various values experimentally obtained have been plotted on a potential-wave-lengths sheet. The curves most nearly fitting the points have been drawn. Their similarity to the schema of the Hering theory is obvious.

In conclusion, we have been desirous of repeating portions of this investigation and of extending the researches to include experimentation upon living animals, thereby hoping to get more definite information upon the conditions of response in living eyes, but the exigencies of the times and the uncertainties surrounding research work have, up to the present, debarred us.

BIBLIOGRAPHY

(1) **Holmgren**: Untersuch. Heidelberg, Bd. ii, 81 and Bd. iii, 308.
(2) **Dewar and McKendrick**: Trans. Royal Soc. Edinburgh, 1872, xxvii, 141.
(3) **Kühne and Steiner**: Physiol. Untersuch. Heidelberg, 1880, iii, 327; 1881, Bd. iv, 564.
(4) **Waller**: Phil. Trans. Royal Soc., B, 1910, cxciii, 123; 1901, B, cxciv, 183; Proc. Physiol. Soc., 1900. Also see his book, The Signs of Life.
(5) **Gotch**: Journ. Physiol., 1903, xxix, 1904, xxxi.
(6) **Einthoven and Jolly**: Journ. Exper. Physiol., 1908, i, 373.
(7) **Fuchs**: Pflüger's Arch., 1894; Bd. lvi, 408; 1901, Bd. lxxxiv, 425.
(8) **DeHaas**: Onderzoekingen Physiol. Lab. Leiden, 2nd ser., vi; also Dissertation Leiden, 1903.
(9) **Piper**: Arch. f. Anat. u. Physiol., Suppl. Bd. 1905, 133.
(10) **Ishihara**: Pflüger's Arch., 1906, Bd., cxiv, 569.
(11) **Bruecke and Garten**: Pflüger's Arch., 1907, Bd. cxx, 290.
(12) **Gotch**: Journ. Physiol., 1904, xxxi, 21.
(13) **Gotch**: Ibid., 1904, xxxi, 26.
(14) **Gotch**: Ibid., 1903, xxix, 410.
(15) **Waller**: The Signs of Life, 34.
(16) **Troland**: Journ. Optical Soc. America, 1917, i, 1.

CHANGES IN THE WEIGHTS OF THE VARIOUS PARTS, SYSTEMS AND ORGANS IN ALBINO RATS KEPT AT BIRTH WEIGHT BY UNDERFEEDING FOR VARIOUS PERIODS

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It has been shown that in young animals stunted by underfeeding certain of the systems and organs of the body show a remarkable tendency to continue their growth, others remain nearly unchanged, while still others lose weight to a variable extent (Aron, '11 and '14; Jackson, '15b; Stewart, '16 and '18a). The object of the present paper is to show the changes which occur in the weights of different organs and systems of the body in albino rats kept at birth weight (about 5 grams) by underfeeding for various periods, and also to compare these changes with those previously observed in larger rats during various periods and degrees of stunting.

Material and methods. In the present experiment 23 albino rats (Mus norvegicus albinus) were used, including 8 newborn controls (5 males, 3 females) and 15 test rats (8 males, 7 females). The sex and body weight for each individual rat is shown in table 1. The letters "St" indicate the series; the number preceding the decimal point indicates the litter to which the rat belongs and the number following the decimal point designates the individual rat.

The test rats were stunted from birth for periods ranging from 11 to 22 days of age (average 16 days) by subjecting them to repeated periods of fasting. This was accomplished by removing the test animals from the mother at successive periods as described in a previous paper (Stewart, '18a). Under normal conditions the body weight of the albino rat at 16 days (the average age of the test rats at the end of the experiment) would have at least trebled the birth weight. Thus the maintenance at birth weight for this period represents a condition of very severe inanition, which is maintained only with great difficulty. Many animals died during the course of the experiment and were not utilized on account of the possibility of post mortem changes. The general plan adopted was to adjust the length of the alternate fasting and nursing periods in such manner as to keep the starved individuals practically at birth weight. In this respect the experiments were very successful for, as is evident from tables 1 and 2, the average weights of the control and test animals are nearly equal.

TABLE 1

Individual data for the albino rats used in the experiments

RAT NO.	USE AND SEX	BIRTH WEIGHT	WEIGHT AT AUTOPSY	AGE
		grams	*grams*	*days*
St 237.2	Control M	4.9	4.9	0
St 246.6	Control M	5.0	5.0	0
St 240.5	Control M	5.1	5.1	0
St 239.5	Control M	5.4	5.4	0
St 242.4	Control M	5.9	5.9	0
Average		5.3	5.3	
St 236.3	Test M	5.0	4.8	12
St 203.6	Test M	4.1	5.0	14
St 240.3	Test M	5.0	5.0	22
St 240.11	Test M	4.9	5.0	21
St 240.7	Test M	4.8	5.0	18
St 236.1	Test M	5.3	5.5	14
St 214.4	Test M	5.5	5.7	14
St 235.8	Test M	5.8	5.8	12
Average		5.0	5.2	16
St 238.1	Control F	5.0	5.0	0
St 240.1	Control F	5.1	5.1	0
St 239.7	Control F	5.5	5.5	0
Average		5.2	5.2	
St 215.5	Test F	5.0	4.0	11
St 235.1	Test F	4.8	4.8	12
St 235.4	Test F	5.0	5.0	12
St 239.10	Test F	5.4	5.0	20
St 214.6	Test F	5.5	5.0	13
St 237.3	Test F	4.1	5.1	18
St 214.2	Test F	4.6	5.1	18
Average		4.9	4.9	15

TABLE 2

Showing the changes in average body length, tail ratio and weights of the various systems and organs in albino rats held at constant body weight by underfeeding from birth to 11 to 22 days of age

	MALES		FEMALES		SEXES COMBINED		AVERAGE PER CENT OF CHANGE
	Controls 5 newborn	8 Underfed 11-22 days	Controls 3 newborn	7 Underfed 11-22 days	Average of controls	Average of test rats	
Gross body weight, grams	5.26	5.22	5.20	4.86	5.24	5.05	−4
Net body weight, grams	5.08	5.03	4.98	4.64	5.04	4.85	−4
Body length, mm	50.4	57.0	50.3	54.7	50.4	55.9	+11
Tail ratio, per cent	31.3	40.5	32.4	43.6	31.7	41.9	+46
Head, grams	1.04	1.55	1.03	1.46	1.04	1.51	+45
Visceral group, grams	0.876	1.340	0.866	1.205	0.873	1.277	+46
Integument, grams	0.758	1.001	0.750	0.881	0.755	0.945	+25
Musculature and skeleton, grams	1.91	2.11	1.86	1.88	1.89	2.00	+6
"Remainder," grams	1.53	0.586	1.51	0.680	1.52	0.630	−59
Testes, grams	0.0027	0.0128			0.0027	0.0128	+374
Epididymis, grams	0.0016	0.0052			0.0016	0.0052	+225
Eyeballs, grams	0.024	0.057	0.023	0.060	0.024	0.059	+146
Brain, grams	0.217	0.525	0.231	0.474	0.223	0.501	+125
Kidneys, grams	0.0416	0.0874	0.0483	0.0794	0.0441	0.0837	+90
Spinal cord, grams	0.035	0.067	0.034	0.061	0.035	0.064	+83
Stomach and intestines (empty) grams	0.151	0.221	0.162	0.212	0.155	0.217	+40
Spleen, grams	0.012	0.019	0.011	0.012	0.012	0.016	+33
Hypophysis, grams	0.00056	0.00071	0.00047	0.00063	0.00052	0.00067	+29
Heart, grams	0.031	0.041	0.031	0.036	0.031	0.039	+26
Pineal body, grams	0.00028	0.00037	0.0003	0.00033	0.00029	0.00035	+21
Stomach and intestines (full) grams	0.334	0.413	0.383	0.427	0.352	0.420	+19
Thyroid, grams	0.00126	0.0015	0.0014	0.0013	0.0013	0.0014	+8
Ovaries, grams			0.00110	0.00116	0.00110	0.00116	+5
Lung, grams	0.088	0.097	0.083	0.079	0.086	0.089	+3
Suprarenals, grams	0.0021	0.0021	0.0017	0.0019	0.0019	0.0020	+5
Liver, grams	0.260	0.199	0.229	0.182	0.248	0.191	−23
Thymus, grams	0.0079	0.0042	0.0079	0.0038	0.0079	0.0040	−49

In comparing the data from the starved individuals with those from the controls, the slight difference in body weight (average about 4 per cent less in the test animals) has generally been disregarded, for the resultant error is small and does not modify the main conclusions based upon the data obtained.

The experiments were made in the Institute of Anatomy, University of Minnesota. The warm room in which the stunted rats were kept while fasting, and also the autopsy technique used are described in a previous paper (Stewart, '18a). The net "body weight" is the gross body weight less the weight of the contents of the alimentary canal.

For economy of space only average data are included in tables 2 and 3. However, copies of the original individual observations will be deposited at the Wistar Institute of Anatomy and Biology, Philadelphia, where they may be consulted if so desired.

Body length and tail length. Immediately after death the body (nose-anus) length and tail length of each rat were measured and recorded. Averages of the individual ratios $\frac{\text{(tail length)}}{\text{(body length)}}$ were computed from the data thus obtained and expressed as percentages (table 2). The ratio of tail length to body length is known as the "tail ratio."

According to the data in table 2, the body length of the rats kept at birth weight for 11 to 22 days (57.0 mm.) averages 11 per cent greater than the body length of the newborn controls (50.4 mm.). This indicates persistent skeletal growth in the young starved rats even though no increase in body weight was permitted. This result agrees with the previous observations by Jackson ('15b) and Stewart ('18a). Continued increase in body length during stunting by underfeeding has been observed not only in the rat, but also in calves (Waters, '08), and in dogs (Aron, '11). Also in malnourished children a strong persistent tendency to growth in body length (skeleton) has been noted by Variot ('07), Birk ('11), Aron ('14), Hess ('16) and others, even in cases where the body weight is stationary. This question is discussed more fully by Jackson and Stewart ('18).

The results (table 2) show the tail ratio to be considerably higher in the test than in the control rats. There is an apaprent increase in the tail length from a normal average of approximately 0.32 (31.7 per cent) of the body length in the newborn controls (sexes combined) to about 0.42 (41.9 per cent) in the starved individuals. This corresponds to an average increase of about 46 per cent in the absolute length of the tail, as compared with an increase of 11 per cent in the body length. It is therefore evident that while the body continues to increase in length even during maintenance at birth weight, the elongation of the tail under these circumstances continues still more rapidly than that of the body, thus producing relatively long-tailed individuals.

Furthermore the data (table 2) show the tail to be relatively longer in the female than in the male rat, not only in the control but also in the test animals. Jackson ('15b) and Stewart ('18a) likewise noted the tail to become relatively long in young rats held at maintenance, and with a persistent tendency to average slightly longer in the female than in the male.

The relative elongation of the tail during underfeeding, although retarded in rate, apparently is an expression of the normal growth process, for according to Jackson ('15b) the tail ratio of the albino rat normally increases from an average of 0.36 (36 per cent of body length) at birth to 0.66 at 3 weeks, and is slightly greater in the female.

Head and body parts. The weight of the head (table 2) averages considerably higher in the test rats of each sex than in the younger controls of corresponding body weight. The combined data indicate an increase from an average of about 1.04 gram in the controls to approximately 1.51 gram in the test rats, an increase of about 45 per cent. As will be evident later, the increase (0.47 gram) can be attributed only partly to an increase in the weight of the brain and eyeballs. The extent to which the additional excess is shared by the integument, skeleton and musculature is uncertain from the data available, for no direct observations were made upon these parts for the head separately.

In older and larger rats underfed for various periods Jackson ('15b) and Stewart ('18a) noted a tendency for the head to increase in the majority of instances, even when the body weight was held constant. In general, however, the changes were not so marked as those observed in the present series of young individuals, in whom the intensity of growth is relatively much stronger. In a group of rats stunted by underfeeding from birth to 3 weeks, (body weight doubled), the average increase in the weight of the head was 16 per cent, as compared with normal controls of the same body weight (Stewart, '18a). This evidence of a relatively stronger growth tendency in the head region in the younger animals recalls the general law of cranio-caudal progression in growth and development, as formulated by Jackson ('09).

No direct observations were made with regard to the weight of the trunk and extremities, but with an increase in the weight of the head during maintenance of constant body weight, the trunk and extremities collectively have necessarily decreased to a corresponding extent.

Visceral group, integument, musculature and skeleton, and "remainder." The weights of the visceral group (including abdominal and thoracic viscera, spinal cord, brain and eyeballs) and of the integument average considerably higher in the test than in the control rats of corresponding body weight, the average increase (sexes combined) amounting to 46 per cent for the viscera and 25 per cent for the integument. Also the musculature and skeleton together show a slight excess weight in the stunted animals in this case, however, amounting to only 6 per cent. The extent to which the change is shared by the skeleton and the musculature individually is uncertain for in this series the two were not weighed separately.

The "remainder" is obtained by deducting the weight of the integument, viscera, skeleton and musculature from the net body weight. It thus includes salivary and lymph glands, tongue, oesophagus, fat and body fluid but not the intestinal contents. A marked loss in weight of the "remainder" has occurred during underfeeding. The data indicate a decrease from an average of 1.52 gram in the controls to 0.63 gram in the test rats of corresponding body weight, a loss of about 59 per cent. The loss suffered by the "remainder" practically compensates for the combined increase observed for the above mentioned systems (correction being made for difference in average body weight).

A review of the literature dealing with the effect of inanition in general upon the parts and systems as well as the individual organs of the body in the rat and other animals is given by Jackson ('15a, '15b) and Stewart ('16, '18a).[1]

Organs gaining weight during fasting. From the data in table 2, it is evident that a considerable number of organs show a remarkable tendency toward continued growth in weight in young rats, even when an increase in body weight is prevented. The organs are arranged in order of their growth intensity when the body is held at birth weight in the first column of table 3. The most striking growth is noted in the case of the testes, epididymis, eyeballs and brain, the increase in the weight of these organs amounting to 374, 225, 146 and 125 per cent respectively. Of the other organs, the kidneys, spinal cord, empty stomach and intestines, spleen, hypophysis, heart and pineal body also increase in weight considerably during maintenance of constant body weight from birth, the average percentages of increase being 90, 83, 40, 33, 29, 26 and 21 per cent respectively. The integument with

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[1] Statements in regard to the "remainder" after long periods of fasting (Stewart, '18a) should be changed to agree with the data, the latter being correct as published. Thus on page 328, line 8, "higher" should be changed to "lower." Also on page 331, line 6, change "six" and "ten" to "three" and "six" (weeks of age, etc.), respectively.

TABLE 3

Changes in weight of organs in underfed albino rats compared with normal at same body weight

	MAINTENANCE FROM BIRTH TO 11-22 DAYS BODY WT. 5 GRAMS. (STEWART)	STUNTING FROM BIRTH TO 3 WKS. BODY WT. 10 GRAMS. (STEWART '18a)	STUNTING FROM BIRTH TO 10 WKS. BODY WT. 15 GRAMS. (STEWART '18a)	MAINTENANCE FROM 3 WKS. TO 10 WKS. BODY WT. 24 GRAMS. (JACKSON '15b)	STUNTING FROM 3 WKS. TO 400 DAYS BODY WT. 50 GRAMS. (STEWART '18a)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Testes	+374	+188	+51	+34	−42
Epididymis	+225	+95	−6	−4	−32
Eyeballs	+146	+41	+66	+50	+73
Brain	+125	+60	+8	−0.5	+4
Kidneys	+90	+21	+38	+4	−6
Spinal cord	+83	+70	+70	+36	+40
Visc. group	+46	+28	+38	+0.9	+1
Head	+45	+16	+4	+6	+26
Stomach and intestines	+40	+17	+100	+28	−27
Spleen	+33	−49	+24	−42	−5
Hypophysis	+29	+33	+24	M +18 F +19	F +10 M −15
Heart	+26	−5	+27	−0.6	−10
Integument	+25	+0	−48	−36	−11
Pineal body	+21	−4	+12		−8
Thyroid gland	+8	+5	+4	−24	−36
Skeleton	+6	+19	+24	+28	+32
Musculature		+8	+10	+3	+4
Ovaries	+5	+83	+54	−27	+17
Suprarenals	+5	+60	+114	M +12 F +39	M +36 F +66
Lungs	+3	−26	−26	−15	+28 (?)
Liver	−23	+17	+64	+10	−7
Thymus	−49	−30	−80	−90	−90
"Remainder"	−59	−40	−23	−1.9	−33

Note. The data in the first column are from table 1.

an increase of 25 per cent and the stomach and intestines including contents with an increase of 19 per cent also belong to this group.

The empty stomach and intestines show an increase from 0.155 gram in the controls to 0.217 gram in the test rats, a gain of about 40 per cent. When the contents are included with the stomach and intestines there is an apparent increase in the starved rats from a normal average of 0.352 gram to 0.420 gram, the increase amounting to about 19 per cent. The smaller gain recorded for the full than for the empty stomach and intestines is evidently due to the fact that the weight of the intestinal contents remained practically unchanged during the underfeeding, increasing from a normal average of 0.197 gram to 0.203 gram in the test rats, a gain of only about 3 per cent.

In the case of the pineal gland the apparent increase of 21 per cent in the test rats may be somewhat questionable, especially when the extremely small size of the organ is taken into consideration. Very small errors in technique of removal and weighing the organ may be responsible for the apparent increase in its weight.

On the whole, however, it is evident that during the severe inanition required to hold the body at birth weight the above mentioned organs continue to increase in weight at the expense of other parts of the body. In some instances the increase accomplished during starvation is very remarkable.

Organs remaining nearly constant in weight during fasting. In a smaller group of organs (see table 2) there is practically no growth in the rats kept at birth weight. The percentage increases (table 3, first column) are as follows: thyroid gland, 8; ovaries, 5; suprarenals, 5; and lungs, 3. The skeleton and musculature, which together show an increase of 6 per cent, also belong in this group. The average weights for these organs differ from those for the control animals by less than 10 per cent although in each case the average small difference indicates a slight apparent increase in weight of the corresponding organ.

Organs losing weight during fasting. Of the organs weighed individually, only two, the liver and thymus (tables 2 and 3) were observed to lose in weight in the rats kept at birth weight. The average data (sexes combined) indicate a loss of 23 per cent for the liver and 49 per cent for the thymus. As previously noted, the greatest loss is found in the "remainder" (decrease of 59 per cent) which, together with that of the liver and thymus, must counterbalance the gain in the other parts of the body since the body weight is held approximately constant.

DISCUSSION AND CONCLUSIONS

On comparing the results of the present experiments with those previously obtained in similar experiments upon older and larger animals a more complete survey than was hitherto available is obtained of the weight changes which occur among the various organs and parts of the body at various ages during inanition. This comparison brings out very strikingly the changing reaction undergone by the various individual organs at different ages when the individual is stunted in growth by underfeeding. The extent and character of this change for individual organs and systems is so well shown in table 3 that a detailed discussion of the individual organs is unnecessary. Special attention is called to certain points, however.

The visceral group as a whole averages 46 per cent increase in weight in the rats held at birth weight, as shown in column 1 of table 3. At later ages, and with longer periods of underfeeding (as shown in the successive columns of table 3) the tendency of the visceral group to increase persists, but becomes weaker. In the rats underfed beginning at the age of 3 weeks (last two columns of table 2), the visceral group barely holds its own in weight. In comparison with this general trend in the visceral group, the individual organs show great variability.

The testes show a remarkable increase of 374 per cent in rats kept at birth weight for various periods. In later periods of underfeeding the tendency to increase in the weight of the testes is progressively weaker, while in rats weighing approximately 50 grams at 400 days a loss of 42 per cent is recorded. Other organs, like the epididymis, brain, kidneys and alimentary canal, during the earlier periods of underfeeding exhibit a marked growth capacity, but later (like the visceral group as a whole) are barely able to maintain themselves, or in some cases actually suffer a loss in weight, under the conditions of the experiment. The eyeballs and spinal cord, however, not only increase markedly in weight in the younger underfed groups, but also tend to maintain strong growth tendencies with considerable persistency, even in the older groups. The thymus at all periods shows a marked loss in weight during underfeeding, while the other organs are variable.

The above mentioned increases in the weights of various organs and parts of the body during underfeeding have been observed to be accompanied also by continued structural development of apparently normal character. Thus in the case of the skeleton (Jackson, '15b and Stewart, '18a) continued growth during stunting has been shown to be accompanied by continued development of apparently normal character (increasing proportion of solids, formation and fusion of epiphyses, development of third molar teeth). A progressive differentiation of the integument and external ear and opening of the eyelids before reaching the body weight at which this normally occurs has also been noted in young rats stunted by underfeeding (Stewart, '18a). The growth of the brain under similar conditions is likewise accompanied by normal differentiation of the parts as shown by the relative weight of the various segments (Stewart, '18b).

While the growth tendencies of the skeleton and musculature are apparently weaker in the present series held at birth weight than in subsequent periods, the integument and the visceral group show decidedly stronger growth capacities than at later periods of underfeeding (see table 3). A relatively enormous growth capacity is exhibited by certain organs during this earliest period. In older and larger rats considerable growth has previously been observed in the case of certain organs during underfeeding, but such remarkable changes as found in the present series (rats kept at birth weight) with increase of 374, 225 and 146 per cent (testes, epididymis and eyeballs respectively) have not hitherto been observed. As a general rule it appears that in most cases the growth tendency of organs during underfeeding is strongest during the youngest stages.

When individual organs are considered, however, it will be noted that there are some exceptions to this rule. For example, the liver shows a loss of 23 per cent in rats kept at birth weight (11 to 22 days) whereas in underfed animals weighing 10 and 15 grams at 3 and 10 weeks of age there is an increase of 17 and 64 per cent respectively. Also in the case of the ovaries and suprarenals there is apparently a lower growth tendency noted in the youngest group as compared with the marked increase found at 3 and 10 weeks of age in rats weighing 10 and 15 grams, respectively. Other organs, such as the spleen and lungs, are somewhat irregular.

In comparing the results of the present experiment with earlier investigations on the effects of underfeeding at later periods, the following generalizations seem justified. The "remainder" suffers a very heavy loss in rats held at birth weight by underfeeding, while the skeleton and musculature show a very feeble gain as compared with the tendency at somewhat later periods. The tendency of continued growth in the integument and visceral group during inanition is stronger in newborn rats than in older and larger animals subjected to similar conditions. This question is discussed in greater detail in the paper by Jackson and Stewart ('18).

The number of individual viscera showing an increase in weight during underfeeding is greater in the young rats than at later periods. While most of the individual organs (like the group as a whole) show a decreasing intensity of growth at later periods of underfeeding, there are several exceptions to the rule, as previously noted.

SUMMARY

1. The weights of the various organs, systems and parts of the body were studied in 8 newborn controls and 15 test rats kept at birth weight by underfeeding for periods ending at 11 to 22 days of age.

2. The underfed newborn rats become relatively long-tailed individuals, as previously found in similar experiments on older animals.

3. During maintenance at birth weight the head increases about 45 per cent in weight, compensated for by a corresponding decrease in the trunk and extremities. This is also similar to the results at later stages.

4. The visceral group increases 46 per cent, the integument 25 per cent and the musculature and skeleton (together) 6 per cent in the test rats, counterbalanced by a corresponding decrease of 59 per cent in the "remainder." The integument and visceral group thus show much stronger growth tendencies than in similar underfeeding experiments at later ages. The musculature and skeleton have apparently weaker growth tendencies than later, while the loss in the "remainder" is relatively greater than at later periods.

5. The individual organs in the test rats, as compared with those in newborn rats of corresponding weight show the following percentage increases in weight: testes, 374; epididymis, 225; eyeballs, 146; brain, 125; kidneys, 90; spinal cord, 83; stomach and intestines (empty) 40; spleen, 33; hypophysis, 29; heart, 26; pineal gland, 21; stomach and intestines (with contents), 19.

In the rats kept at birth weight the thyroid gland, ovaries, lungs and suprarenals remain practically unchanged in weight (gain of 8, 5, 5 and 3 per cent respectively); whereas the liver and thymus suffer a loss of 23 and 49 per cent respectively.

6. These changes in rats held at birth weight by underfeeding resemble in general those previously found in somewhat larger and older stunted animals (Jackson, '15b; Stewart, '18a; Jackson and Stewart, '18). Certain differences appear, however, as shown in table 3.

BIBLIOGRAPHY

Aron, H. '11. Philippine Journ. Sci., vi, 1.
'14. Berl. klin. Wochenschr., li, 972.
Birk, W. '11. Berl. klin. Wochenschr., Bd. ii, 1227.
Hess, A. F. '16. Amer. Journ. Diseases Children, xii, 152.
Jackson, C. M. '09. Amer. Journ. Anat., ix.
'15a. Amer. Journ. Anat., xviii. Also abstracted in Anat. Rec., ix, 90.
'15b. Journ. Exper. Zoöl., xix. Also abstracted in Anat. Rec., ix, 91.
Jackson, C. M. and C. A. Stewart. '18. Minnesota Medicine, i.
Stewart, C. A. '16. Biol. Bull., xxxi, 16. Also abstracted in Anat. Rec., x.
'18a. Journ. Exper. Zoöl., xxv. Also abstracted in Anat. Rec., xi, 410.
'18b. Journ. Exper. Neurol.
Variot, G. '07. Bull. et Mém. Soc. méd. d. hôpit. de Paris, xxiv, 1347. Also in Bull. soc. de pediatr. de Paris, ix, 363.
Waters, H. J. '08a. Proc. Soc. for the Promotion of Agric. Sci.
'08b. Presented at the 30th Meeting Soc. for the Promotion of Agric. Sci.

THE STIMULATION AND INHIBITION OF THE GASTRIC SECRETION WHICH FOLLOWS THE SUBCUTANEOUS ADMINISTRATION OF CERTAIN ORGAN EXTRACTS

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In a previous communication (1) we showed that the subcutaneous injection in dogs of saline extracts of several different organs was followed in each case by an increase in the gastric secretion. Extracts made from the adrenal and pituitary glands alone, of those tested, inhibited the gastric secretion both before and after the injection of the stimulant extracts.

These extracts were made by shaking for two hours the hashed fresh organ in normal salt solution which had been made slightly alkaline with NaOH. This was then stored in the ice box for twenty-four hours. After straining off the coarser particles through gauze and filtering through paper, enough 10 per cent acetic acid was added to the opalescent filtrate to precipitate the nucleo-proteins. After their removal the filtrate was boiled and again filtered to separate the acid albumins and this filtrate rendered faintly alkaline to litmus was, also boiled and filtered. This final slightly alkaline liquid then contained only the non-coagulable portion of the original saline extract and for convenience was designated as the "residue" of this or that organ. The injection into dogs of the "residue" of each of the endocrine glands was followed by some definite and immediate physiological reaction which seemed traceable to either stimulation or inhibition of a part of the involuntary nervous system.

In the case of the thyroid, none of the coagulable materials in its saline extract were found capable of producing any demonstrable reactions.

For the adrenal, however, further experimentation has shown that almost as vigorous responses could be excited in the stomach by the adrenal nucleo-proteins as by the adrenal "residue." The latter is rich in epinephrin while the nucleo-proteins contain only traces of this substance.

In the following article we have confined ourselves to the study of the effects upon the gastric secretion of the subcutaneous injection of extracts of the pituitary and adrenal glands and more particularly of the thyroid. We had previously employed dogs in which the usual Pawlow gastric pouch and fistula had been established, but as this operation seemed liable to damage the nerve supply of the pouch, we have employed in these experiments the Janeway* fistula. A trap door flap about 2 inches square, with its base at the greater curvature, is cut from the anterior surface of the stomach. The flap is turned back and folded together by a continuous suture which unites the right and left borders of the flap and then the right and left edges of the gap in the stomach. The open extremity of the resultant artificial tube, which is continuous with the interior of the stomach, is then brought out through a stab wound in the left rectus and sutured to the skin. This fistula does not leak and after the wounds have healed, readily allows the passage of a catheter for obtaining the gastric secretion.

During the tests the animals have been placed in a retaining frame in which they can be kept quiet for an hour or more. Our dogs were given no food for at least twelve hours before the test. To make them react satisfactorily the animals must be maintained in the best possible condition.

Disease or malnutrition very evidently interferes with the amount of gastric secretion and with the responsiveness of the stomach to the test materials. The thyroid extracts were standardized according to both their iodine and nitrogen content, and the adrenal and pituitary extracts according to their nitrogen content alone. The injections were made with every aseptic precaution, usually into the subcutaneous tissue at the back of the neck. Then, with the dog standing in the retaining frame, a catheter was introduced into the fistula and the gastric secretion collected during successive fifteen-minute periods.

A great number of tests were made and table 1 shows a fair average of the results for the different preparations from the thyroid. It was found to be difficult or impossible to use the same dog for each test because a twenty-four-hour rest period, at the least, had to be allowed to maintain the animals in good condition. This often had to be much extended. The feeding took place in the afternoon, and the morning hours, when the stomach contains no food, were used for the experiments. In the second column of table 1 are noted the (normal) quantity in cubic centimeters of gastric juice, collected during the first fifteen minutes and its total acidity expressed as HCl. This was determined by titration against $\frac{N}{10}$ NaOH, with phenolphthalein as the indicator. In the next column are noted the thyroid materials and the amount injected expressed in terms of its iodine and protein content. The next three columns represent the quantity and total acidity of the gastric juice collected during the three fifteen-minute intervals which follow the brief period required by the experimenter to make the test injection and then note the time. That is, about five minutes elapse between the close of the collection of the normal flow and the beginning of the collection of the test flow.

As remarked before, we had previously determined that the "residue" of a saline extract of the thyroid of any given animal, when standardized by its iodine content, was quite uniformly active. Of the common abattoir animals, the pig thyroid contains the largest amount of iodine, the sheep contains much less and the beef, the least iodine of the three. Incidentally it should be noted that sheep thyroids are very often goitrous and hence have an extremely low iodine content, and therefore care must be used to select only normal glands. Their iodine content, as is well known, shows a considerable seasonal variation, being the richest during the spring and summer months.

The first test in table 1 shows the comparative activity upon the stomach of the "residue" or non-coagulable portion of saline extracts of the pig, sheep and beef thyroids. The next tests show the comparative activity of pig thyroids, which were obtained from the abattoir in as *fresh* a condition as possible, and immediately extracted, and of those which had been kept before extraction, in a *frozen condition for about two weeks;* and finally, of the activity of an extract or "residue," made in exactly the same way as the others, from the commercial desiccated thyroid powder, which presumably consists of sheep thyroid glands.

As the active material in our "residues" is non-coagulable, we next tested alcoholic and ethereal extracts of the pig thyroid. The hashed glands were shaken for two hours in ether or 95 per cent alcohol, and stored for several days at room temperature, in corked bottles. The alcoholic or ether extract was filtered so that we obtained a clear liquid and then the alcohol or ether distilled off until only about one-eighth to one-tenth of the original volume remained. This was then diluted with distilled water and tested as to iodine and nitrogen content. A similarly prepared 95 per cent alcohol extract of commercial desic-

TABLE 1

Effects upon the flow of gastric juice and its acidity, which follow the injection of thyroid and other "extracts"

DOG NO.	NORMAL FLOW FIRST 15 MINUTES AMOUNT HCl		MATERIAL INJECTED	AMOUNT	EFFECT DURING SECOND 15 MINUTES AMOUNT HCl		THIRD 15 MINUTES AMOUNT HCl		FOURTH 15 MINUTES AMOUNT HCl	
	cc.	*per cent*			*cc.*	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*
XX	3.0	0.46	Pig thyroid residue	7.0 cc. = {0.2 mgm. I 0.19 gram Prot.	21.5	0.52	21.0	0.54	10.0	0.54
XX	1.5	0.4	Sheep thyroid residue	10.0 cc. = {0.125 mgm. I 0.25 gram Prot.	6.5	0.52	6.0	0.48	5.0	0.48
XXII	3.4	0.36	Beef thyroid residue	2.8 cc. = {0.028 mgm. I 0.1 gram Prot.	4.0	0.44	3.0	0.4	2.0	0.4
XXIX	5.0	0.33	Pig thyroid residue "fresh" glands	4.0 cc. = {0.1 mgm. I 0.4 gram Prot.	13.0	0.33	10.0	0.45	9.0	0.44
XXIX	1.0	0.4	Pig thyroid residue frozen 2 weeks	4.0 cc. = {0.1 mgm. I 0.4 gram Prot.	12.0	0.5	13.0	0.5	8.0	0.45
VIII	3.4	0.36	Sheep (?) thyroid residue from commercial desiccated powder	2.5 cc. = {0.2 mgm. I 0.075 gram Prot.	7.0	0.36	6.0	0.38	4.0	0.34
XXIX	4.0	0.5	95 per cent alcohol extract pig thyroid	3.0 cc. = {0.033 mgm. I. 0.3 gram Prot.	15.0	0.65	16.0	0.6	14.0	0.6
XXIX	2.0	0.36	Ether extract pig thyroid	2.0 cc. = {Trace I 0.1 gram Prot.	4.0	0.43	5.5	0.46	4.0	0.46

XIV	4.0	0.28	95 per cent alcohol extract desiccated commercial (sheep) thyroid	2.8 cc.=	0.15 mgm. I 0.1 gram Prot.	8.0	0.36	7.0	0.3	7.0	0.3
III	2.6	0.3	Distilled water extract pig thyroid	5.0 cc.=	0.08 mgm. I 0.1 gram Prot.	1.0	0.36	1.0	0.36	Few drops	
VIII	2.6	0.3	Solution of Kendall's preparation from pig thyroids	2.1 cc.=	0.1 gram Prot.	1.5	0.28	1.5	0.28		
III	1.5	0.4	Beef muscle residue	4.0 cc.=	0.1 gram Prot.	1.5	0.4	1.0	0.4		
XXX	Few drops		Edkin's "gastrin"	7.0 cc.=	0.1 gram Prot.	8.0	0.5	11.0	0.58	5.0	0.5

cated thyroid powder (presumably from sheep glands) was also tested. An extract, according to Kendall's method (2) published in 1915, was made and tested. It had a high nitrogen and iodine content.

To show that a "residue" of any tissue is not active there is inserted a test made with the non-coagulable part of a saline extract of beef muscle. In the last line there is recorded for comparison a test made with Edkin's "gastrin" (3).

From the above figures it can be seen that a dose of the pig, sheep or beef thyroid which contains approximately the same amount of protein, varies considerably in the amount of iodine. And the greater the amount of iodine, the greater is the stimulant effect. The "residue" of the pig thyroid increases the gastric flow usually about sevenfold, of the sheep thyroid about fourfold, while the beef gland shows only slight activity. The total acidity is regularly increased but not to any such extent as is the quantity of gastric secretion.

There is a general belief that glands obtained in as "fresh" a condition as possible are preferable to, or more active than, those which have been stored for days or weeks, in a frozen state. In all our tests the frozen glands were fully as active as the "fresh." This is a matter of considerable importance as glands in quantity, for commercial purposes, practically must be stored for a greater or less length of time. The "residue" or alcohol extract of the common desiccated powder of sheep thyroid glands is only about half as active as corresponding preparations from fresh glands. Extracts of the dried material also show an increase in the protein content with a decrease in iodine, and this circumstance combined with its demonstrably lessened activity indicates some deterioration in quality.

An alcohol extract of the thyroid contains a little less iodine and more protein than our "residue" and is somewhat less active, and the average of a number of tests of comparable doses of the alcohol and saline or "residue" extracts shows that their stimulant powers upon the stomach are much in favor of the "residue." An ether extract consists almost entirely of fatty material which for injection has to be made into an emulsion with distilled water. This contains only traces of iodine but shows considerable activity. An ether-alcohol extract shows approximately the same activity as one made with alcohol alone.

The injection of a solution of Kendall's thyroid "active principle," as described above was followed by no response. An extract of pig thyroid made with distilled water was proved by repeated tests to be entirely inert. Thus evidently indicates that hydrolysis is necessary before the active thyroid material can be obtained in watery solutions. Tests with a "residue" made from beef muscle prove only that hydrolyzed extracts of these organs have no stimulant effect upon the stomach, but the observation is inserted to show that "residues" of all tissues are not gastric activators.

The normal human thyroid contains approximately the same amount of iodine as the pig gland, or about 0.3 mgm. per gram of fresh tissue, and a "residue" or alcohol extract of this is closely comparable in its effects upon the stomach to a similar extract from the pig thyroid. The human organ in an individual who shows the symptoms of hyperthyroidism, is usually enlarged symmetrically, and is of even consistency throughout, in which case the infolding and proliferation of the alveolar epithelium characteristic of its supposed overactivity occurs more or less abundantly according to the severity of the symptoms, and in pronounced cases involves the entire diseased gland. Or it may be asymmetrically enlarged and of uneven consistency, in which case there are usually one or more encapsulated adenomatous or cystic or mixed cyst-adenomatous masses with the characteristic hyperthyroid changes surrounding, but not in the tumors. From these solid adenomata and from typical hyperthyroid glands, obtained fresh from operations, extracts were made as from the pig thyroids and the results recorded in table 2.

It will be noted that the first three tests are recorded from extracts from "human goiter." These were solid adenomata obtained from patients who presented moderate symptoms of hyperthyroidism, but the histology of the excised tissue showed only simple adenoma. The two last tests were each made with extracts of portions of glands containing the characteristic hyperthyroid changes and were obtained from patients suffering from typical exophthalmic goiter. Many other tests were made with extracts from similarly diseased thyroids, but like these, all were uniformly negative. Unfortunately there was seldom enough tissue to make both an iodine and a nitrogen determination, and as these experiments were carried out as material could be obtained, while tests with other organ extracts were in progress, only the protein content of the dose was ascertained. This permitted comparisons with organ extracts other than the thyroid, but there was never any response. That is, the symptoms of hyperthyroidism are apparently not *directly* due to an excessive quantity of thyroid product. Clinically the severity of the disease certainly does not seem to bear any relation to the size of the gland. Neoplastic epithelia are not

TABLE 2

Effects upon the flow of gastric juice and its acidity, which follow the injection of extracts from pathological human thyroids

DOG NO.	NORMAL FLOW FIRST 15 MINUTES AMOUNT HCl		MATERIAL INJECTED	AMOUNT	EFFECTS DURING SECOND 15 MINUTES AMOUNT HCl		THIRD 15 MINUTES AMOUNT HCl		FOURTH 15 MINUTES AMOUNT HCl	
	cc.	*per cent*			*cc.*	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*
XV	3.0	0.21	'Residue' of simple human goiter	2 cc. = 0.19 gram Prot.	2.0	0.24	1.5	0.2	Few drops	
XVIII	8.0	0.27	'Residue' of simple human goiter	7 cc. = 0.1 gram Prot.	5.2	0.36	5.0	0.36	2	0.3
XIII	5.0	0.22	95 per cent alcohol extract simple human goiter	12 cc. = 0.1 gram Prot.	3.6	0.2	2.7	0.2		
XV	4.1	0.18	95 per cent alcohol extract hyperthyroid human gland	10 cc. = 0.4 gram Prot.	2.5	0.36	Few drops			
VIII	8.0	0.37	Residue of human hyperthyroid gland	7 cc. = 0.1 gram Prot.	5.2	0.36	5.0	0.36		

generally believed to produce any secretion and the above experimental findings with thyroid adenomata seem to confirm this belief.

The lack of response from extracts of thyroids which showed active hyperplasia at least indicates so great a change in the quality of the product as to prevent the fulfillment of its usual function.

We next tested the effects upon the stomach of the subcutaneous injection of atropin, nicotine, commercial adrenalin and extracts of the adrenal and pituitary glands. In previous experiments (1) as remarked before, we had ascertained that the adrenal and pituitary were the only organs which seemed capable of inhibiting the gastric secretion. Nicotine was accidentally noted to have this effect and although it is understood to act upon some intermediate nervous mechanism it was employed as a comparison with atropin, which paralyzes the terminal filaments of the vagus.

The records given above are a fair average of a large number of tests. It is well known that the secretory activity of the stomach is very largely controlled by the vagi, stimulation of which increases the flow, and by the sympathetic which inhibits. It can be noted that a moderate dose of atropin very quickly stops the gastric secretion, presumably by paralysis of the terminal filaments of the vagus. Nicotine was tested in both large and minute doses and seemed to exhibit inhibitory powers somewhat less than atropin; the inhibition was about in proportion to the size of the dose. Certainly no stimulation could be detected after the administration of even very minute amounts.

Epinephrin is generally accepted as acting through some stimulant effect upon the terminal filaments of the sympathetic, and thus should inhibit the gastric secretion. The typical test with a 1:1000 solution of adrenalin hydrochloride recorded in the third line of table 3, shows that a moderate dose cuts down the normal flow of gastric juice about one-half. A similar dose of a "residue" or an alcohol extract of the adrenal, which contains considerably more epinephrin, suggests that these materials, because of their epinephrin content, act in proportion to it by stimulation of the terminal filaments of the sympathetic. But the injection of a comparable quantity of the adrenal nucleo-proteins, which contain only faint traces of epinephrin is as active or more so than the adrenal "residue" or the alcohol extract or commercial adrenalin. Presumably its effects upon the secreting gastric epithelium is like adrenalin and exerted through the intermediation of the terminal sympathetic filaments. But if it is even more active than adrenalin this fact arouses a suspicion that the active principle of the adrenal gland may contain something more than epinephrin. That is, epinephrin which undoubtedly exists in the adrenal, may be only the stable part of a larger and more labile molecule.

The final three tests with extracts, first of the whole pituitary gland and then with similar preparations from its anterior and posterior lobes, show only that the anterior lobe of this gland seems to exert some inhibitory influence upon the gastric secretion.

TABLE 3

Effects upon the flow of gastric juice and its acidity, which follow the injection of atropin, extracts of the adrenal and pituitary glands, and of nicotine

DOG NO.	NORMAL FLOW FIRST 15 MINUTES AMOUNT HCl		MATERIAL INJECTED	AMOUNT	FLOW DURING SECOND 15 MINUTES AMOUNT HCl		THIRD 15 MINUTES AMOUNT HCl	
	cc.	*per cent*			*cc.*	*per cent*	*cc.*	*per cent*
VIII	4.1	0.3	Atropin sulf.	15 mgm.	0.5	0.4	Few	drops
VII	2.2	0.2	Nicotine	1 mgm.	Few	drops	Few	drops
IX	6.0	0.65	1:1000 solution adrenalin chloride	3 cc.	3.0	0.65	2.0	0.65
VIII	3.1	0.33	Adrenal "residue"	3.0 cc. = 0.1 gram Prot	0.2	0.35	Few	drops
XI	2.6	0.53	95 per cent alcohol extract adrenal	6.3 cc. = 0.076 gram Prot.	0.5	0.44	0.15	0.63
XI	4.0	0.28	Adrenal nucleoprotein	4 75 cc. = 0 076 gram Prot.	0.1	?	Few	drops
XI	2.4	0.38	Pituitary residue	1.26 cc. = 0 076 gram Prot	1.0	0.36	0.9	0.38
VI	2.6	0.19	Residue anterior lobe pituitary	2 75 cc = 0 076 gram Prot.	0.9	0.14	0.8	0 1
V	1.5	0.3	Residue post. lobe pituitary	3.4 cc. = 0.1 gram Prot.	1.8	0.35	1.4	0.37

In table 4 are recorded the average results of a large number of tests to show first the stimulant effect which follows the injection of the thyroid "residue," and the effect upon this stimulation which follows the injection of *a*, atropin to paralyze the vagus; *b*, of adrenalin to stimulate the antagonist of the vagus or the sympathetic; *c*, of the adrenal "residue" which contains much epinephrin and presumably acts like the 1:1000 solution of adrenalin; *d*, of the alcohol extract of

TABLE 4

Effects which follow the injection of atropin, extracts of the adrenal and pituitary glands, and nicotine, after the injection of thyroid material has stimulated the flow of gastric secretion

DOG NO.	NORMAL FLOW FIRST 15 MINUTES AMOUNT HCl		STIMULATION BY	AMOUNT INJECTED	FLOW DURING SECOND 15 MINUTES AMOUNT HCl		INHIBITION BY	AMOUNT	FLOW DURING THIRD 15 MINUTES AMOUNT HCl		FOURTH 15 MINUTES AMOUNT HCl		LINE NO.
	cc.	*per cent*			*cc.*	*per cent*			*cc.*	*per cent*	*cc.*	*per cent*	
VIII	4.0	0.25	Thyroid residue	5.2 cc. = 0.1 gram Prot.	17.0	0.38	Atropin sulf.	15 mgm.	6.0	0.38	2.2	0.3	1
XVIII	2.0	0.18	Thyroid residue	4.0 cc. = 0.1 gram Prot.	16.0	0.27	Atropin sulf.	15 mgm.	9.4	0.42	9.0	0.48	2
XVII	1.5	0.12	Thyroid residue	4.0 cc. = 0.1 gram Prot.	8.0	0.2	1:1000 solution adrenalin chloride	1.5 cc.	5.0	0.3	1.0	0.36	3
III	1.0	0.36	Thyroid residue	3.7 cc. = 0.1 gram Prot.	12.0	0.55	Adrenal residue	3.0 cc. = 0.1 gram Prot.	1.1	0.53	4.8	0.53	4
I	1.4	0.36	Thyroid residue	3.7 cc. = 0.1 gram Prot.	7.5	0.47	Adrenal nucleoproteins	6.2 cc. = 0.1 gram Prot.	1.2	0.42	0.15	0.32	5
III	0.2	0.2	Thyroid residue	3.7 cc. = 0.1 gram Prot.	4.6	0.25	95 per cent alcohol extract adrenal	8.3 cc. = 0.1 gram Prot.	1.6	0.21	1.8	0.27	6
VII	0.2	0.2	95 per cent alcohol extract thyroid	2.0 cc = 0.1 gram Prot	7.4	0.24	Pituitary residue	2.0 cc. = 0.1 gram Prot	4.0	0.21	3.0	0.27	7
VIII	4.8	0.28	Thyroid residue	4.0 cc. = 0.1 gram Prot.	16.2	0.38	Nicotine	0.75 gram	11.0	0.36	7.5	0.38	8

the adrenal which is closely similar to the adrenal "residue;" *e*, of a solution of the adrenal nucleo-proteins which contains only traces of epinephrin; *f*, of the pituitary "residue," and finally *g*, of nicotine.

After stimulation of the gastric flow by the injection of the non-coagulable or "residue" part of a thyroid extract, paralysis of the vagus by atropin only checks the secretion. Atropin does not then completely stop the flow as it does in the unstimulated stomach. Adrenalin, on the other hand, which "activates" the sympathetic or inhibitory nerve supply of the stomach, only checks the *unstimulated* flow, and shows about the same amount of inhibition upon the stimulated secretion. The injection of the adrenal "residue" or of an alcohol extract of the adrenal, which are both rich in epinephrin, is followed by about the same decrease in the flow of the unstimulated, but a considerably greater decrease in the stimulated gastric secretion.

The injection of a solution of the adrenal nucleo-proteins which contains only traces of epinephrin, is followed by a similar and even a slightly more vigorous inhibition. These facts seem to indicate that the gastric nerves are very important, if not essential, for the stimulation produced by the thyroid and for the inhibition by the adrenal. Apparently also the thyroid acts through or upon the vagus and not, as is generally believed, upon the sympathetic. The inhibitory powers of the latter nerves are apparently intensified by some substance produced by the adrenal gland, in which epinephrin forms a part. This substance is probably not epinephrin alone, because adrenalin does not act as vigorously as do extracts of the whole adrenal gland, or the adrenal nucleo-proteins, in which, as stated previously, there are only traces of epinephrin (table 4, lines 3, 4 and 5).

The non-coagulable or "residue" part of a saline extract of the pituitary is the only other organ derivative which we have found to possess inhibitory powers over the flow of gastric secretion. It is interesting to note that extracts of the posterior lobe seem inert. The anterior only is active, but an extract of the whole gland proved more active than one of the anterior lobe alone. Nicotine is supposed to paralyze not the terminal filaments of the sympathetic, but an intermediate portion of the system. It was employed in these tests because it was unexpectedly found to inhibit the gastric secretion in more or less direct proportion to the size of the dose.

As yet we can offer no very definite explanation for these phenomena. Atropin quite evidently paralyzes and so stops the functionation of the "activating" or autonomic portion of the involuntary nervous system.

It is also evident that the adrenal product has a selective affinity for the terminal filaments of the sympathetic, as distinguished from the autonomic or vagus group of nerves. The thyroid product, on the other hand, seems to possess at least some of the same kind of affinity or stimulant power for the opposing or vagus group of filaments. It certainly does not stimulate the sympathetic, or its effect upon the stomach would be intensified and not decreased by the injection of either atropin or adrenalin.

Ergotin is supposed to paralyze the terminal filaments of the sympathetic, but in our experiments upon the stomach it was found to be so variable and uncertain as to be useless. Our extracts of the whole adrenal gland all contain at least traces of epinephrin, and therefore presumably act like it, i.e., through or upon the sympathetic. This, of course, brings into question epinephrin. Is it the normal active principle of the gland or only the stabile portion of a larger and more labile product? That cannot be answered from these experiments, although it is quite evident that extracts of the whole gland show a greater inhibitory effect upon the gastric secretion than does adrenalin.

DISCUSSION

The thyroid gland evidently stimulates, and the adrenal inhibits, gastric secretion. These effects are apparently produced chiefly through the intermediation of the vagus and sympathetic nerve terminals. The secretory functions of the vagus are apparently excited by some material which can be extracted from the thyroid by alcohol or by a process which involves more or less hydrolysis of the gland. The inhibitory powers possessed by the sympathetic terminals seem to be excited by all the extracts from the adrenal gland which were tested. Extracts from the entire gland are much more active than adrenalin. Can this activation or inhibition of the secretory cells of the stomach through their nerve supply in conjunction with thyroid and adrenal products be a process which is essentially one of nutrition? A prolonged stimulation like that produced by the thyroid extracts, when compared with the increased general metabolism which follows thyroid feeding, suggests that the thyroid product, with the intermediation of the vagus or secretory nerve impulse, increases the metabolism of the gastric epithelium. This means that it facilitates the absorption of nutritional material or "food" by the cells and the metamorphosis of this "food" into the cell's secretion. The adrenal product, on the other hand, in conjunction with the sympathetic or inhibitory nerve impulse, can be imagined as preventing this metamorphosis. The nature of the impulses conveyed to the stomach by its double nerve supply is, of course, unknown but, as determined by electrical stimulation, the vagus evidently activates and the sympathetic inhibits this organ If the thyroid product has an affinity for the vagus terminals and promotes cell metabolism, and the adrenal an affinity for the sympathetic and retards this metabolism, then it is unnecessary to imagine two radically different kinds of nerve impulses. The chemical properties of the nerve endings are alone to be considered. The nature of the impulse in the vagus and the sympathetic can be the same, but the effects of its discharge from one nerve terminal or the other are regulated by the presence and amount of thyroid or adrenal, and probably many other products.

CONCLUSIONS

1. A slightly alkaline saline solution, or alcohol, extracts from the thyroid some non-coagulable material which is a vigorous stimulant for the gastric secretion.
2. This material produces its effects at least in part by intensification of the functions performed by the terminal filaments of the (gastric) vagus.
3. Extracts similarly made from the pathological tissue of adenomatous or hyperthyroid human glands are inert.
4. Extracts of the adrenal gland vigorously inhibit gastric secretion.
5. These extracts of the adrenal gland all contain more or less epinephrin and therefore it is presumable that their effect is produced by intensification of the inhibitory function which is ascribed to the (gastric) sympathetic.
6. Adrenalin is not as active a gastric inhibitor as the adrenal nucleo-proteins obtained from extracts of the whole gland. These adrenal nucleo-proteins contain only traces of epinephrin.
7. Extracts of the pituitary gland also inhibit gastric secretion, but only about one-half as vigorously as do extracts of the adrenal.

BIBLIOGRAPHY

(1) Rogers, Rahe, Fawcett and Hackett: This Journal, 1916, xxxix, 345.
(2) Kendall: Journ. Amer. Med. Assoc., 1915, lxiv, 2042.
(3) Edkins: Journ. Physiol., 1906, xxxiv, 133.

QUANTITATIVE STUDIES ON THE RATE OF RESPIRATORY METABOLISM IN PLANARIA

I. The Effect of Potassium Cyanide on the Rate of Oxygen Consumption

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INTRODUCTION

For the study of energy relations in organisms, the lower animals offer certain advantages over the higher vertebrates. Such regulatory mechanisms as those controlling the ventilation of the lungs, the rate of flow of the blood, the oxygen-carrying power of the blood and the maintenance of constant body temperature, which impose limitations upon experimentation with higher vertebrates, can be avoided by the selection of proper material from among invertebrates. Planaria possess none of these mechanisms and they are well fitted for such studies because they can withstand prolonged starvation or can ingest a large meal, and they have remarkable powers of regeneration and agamic reproduction. Experiments upon Planaria have played a prominent rôle in the formulation of theories by Child (1) regarding the so-called "rate of metabolism" in relation to senescence, rejuvenescence and dominance in organisms. Child used the susceptibility of animals and parts of animals to the toxic action of KCN as a measure of the "rate of metabolism." This use of cyanide was based upon the belief of many physiologists that it inhibits oxidations in organisms and upon certain qualitative tests in which, it is reported, the carbon dioxide production by worms in Tashiro's "biometer" agreed with what was expected from their susceptibility to cyanide. No quantitative studies of the metabolism of Planaria by direct methods of measurement have been published heretofore. The author has undertaken a series of such studies for the purpose of determining whether KCN can be used to measure the rate of oxidation in these animals and to determine the important facts regarding the rate of oxidation in Planaria in relation to nutrition, age and morphogenesis. These studies were suggested by Prof. E. J. Lund, under whose direction they have been carried out and to whom I wish to express my most sincere appreciation for assistance and encouragement.

The present paper deals with methods of measuring the oxygen consumption by Planaria and the effect of KCN upon the oxygen consumption by these animals.

The belief that cyanides inhibit intracellular oxidations finds support in a number of studies of the respiratory exchange of organisms, beginning with the classical studies of Geppert (2). Schroeder (3) reported a marked decrease in the oxygen consumption by the mold, Aspergillus niger, in KCN solutions. Warburg (4) and Loeb and Wasteneys (5) agree that the oxygen consumption by sea urchin eggs is decreased very strongly in cyanide solutions; and Loeb and Wasteneys (6) have shown a similar effect of cyanide on the oxygen consumption by Fundulus embryos and by the jellyfish, Gonionemus. Experiments of Hyman (7) also tend to show a decrease in the oxygen consumption by marine sponges in KCN, although there may be question regarding the rate of flow of water through the sponge in cyanide solutions in these experiments. On the other hand Lund (8) has found that the oxygen consumption by Paramecium is independent of the action of KCN.

In non-living systems various authors since Schonbein (9) have found that cyanides inhibit the activity of oxidizing enzymes, but in some cases the cyanide has an opposite effect. Thus Kastle and Loevenhart (10) report that HCN inhibits the oxidations of guaiacum by potato oxidase and many inorganic oxides and peroxides, but that it accelerates the oxidation of guaiacum by silver oxide. Loevenhart (11) found that HCN retards catalysis of hydrogen peroxide by platinum black, silver and extract of tissues, but that it accelerates catalysis of hydrogen peroxide when copper or iron and its salts are used. And the same author (12) finds that HCN accelerates the catalyzing action of copper sulphate upon the oxidation of formic acid by hydrogen peroxide. Matthews and Walker (13) find that HCN prevents the oxidation of cystein to cystin but (14) that it accelerates the oxidation of impure cystin or cystin solutions containing ferric chloride, and that it has no effect on the oxidation of pure cystin.

These results lead to caution about predicting that KCN inhibits oxidations in every system. Are the oxidation systems in all organisms alike in respect to KCN or are there some oxidation systems like the oxidation of cystein and other systems like the oxidation of cystin? May there be other animals than Paramecium whose respiratory exchange is not affected by cyanides? In determining the value of KCN as a measure of the rate of oxidations in any form it is necessary, first of all, to determine its effect upon the oxidations in that form. It has not been shown, heretofore, that KCN affects the oxidations in Planaria.

METHODS

The materials used in this investigation were Planaria maculata, collected from the Mississippi River, and Planaria agilis, purchased and reared in the laboratory. The latter species has been found more convenient for experimentation and most of the experiments reported in this paper were performed with this species. Worms of the former species cling to the substratum so tenaciously that it is often necessary to loosen them with a probe. Not only is this inconvenient but it introduces the danger of injuring the animals.

In each experiment the animals selected were as nearly uniform in size as possible, and had not recently regenerated the head. All animals used in a single experiment were taken from the same stock, having the same history for some days previous to the experiment. The nutritive history and the relative size of worms used in each experiment are stated in the heading of the table reporting the results of the experiment.

The general method employed for determining the oxygen consumption was to place worms in tap water of known content of dissolved oxygen, and at the end of a stated period of time to remove the worms and analyze the water for dissolved oxygen, and so to determine by difference the amount of oxygen consumed. The general procedure was similar to that described by Lund (15) for determining the oxygen consumption by Paramecium, except that the worms were removed from the bottles at the end of the experiment, before the reagents were added, thus eliminating any errors due to absorption of iodine by the organisms during the analysis.

Reagents for Winkler's method of analysis were made up as given by Sutton (16, p. 285) except that 2 cc. of concentrated hydrochloric acid were used as given by Birge and Juday (17, p. 13). The thiosulphate solution was standardized at intervals against known weights of freshly resublimed iodine.

A large carboy was filled with the tap water to be used in an experiment. When the tap water was much warmer or cooler than the room temperature it was allowed to stand in the carboy for twenty-four hours or longer to reach the room temperature. The water was thoroughly stirred and brought into approximate equilibrium with room air as to its dissolved oxygen by drawing a stream of air through it for about two hours. The bottles used in the experiment were then all filled in rapid succession by siphoning water from the carboy to the bottom of the bottles, allowing them to overflow about three times their volume of water. All bottles had fitted ground glass stoppers and were of the same capacity. Bottles which had originally 1 or 2 cc. more than 132 cc. capacity contained pieces of glass rod to make them uniform with the others. The degree of uniformity of filling and analysis is illustrated by table 1, which shows the result of filling the whole series of 28 bottles with aerated tap water and analyzing them at once. The analyses ranged from 4.52 cc. to 4.60 cc. thiosulphate. The average of all analyses was 4.56 cc. thiosulphate. The maximum error in an individual analysis, therefore, was about 1 per cent of the total oxygen content of a bottle, and since duplicate determinations were made in all experiments, the error in filling and analysis was reduced even below this figure.

TABLE 1

Showing the degree of uniformity of filling and analysis of 28 bottles filled in order with aerated tap water and analyzed at once

CC. OF THIOSULPHATE	CC. OF THIOSULPHATE	CC. OF THIOSULPHATE	CC. OF THIOSULPHATE
4.55	4.56	4.53	4.58
4.59	4.55	4.57	*4.52*
4.56	4.56	*4.60*	4.55
4.52	4.54	4.58	4.59
4.57	4.55	4.58	4.56
4.58	4.56	*4.52*	4.57
4.57	4.55	4.56	4.53

Maximum	4.60 cc. thiosulphate
Minimum	4.52 cc. thiosulphate
Average	4.56 cc. thiosulphate

The bottles needed in an experiment were filled in order, as explained above. Then three or four bottles, selected from different parts of the series, were set aside as "blanks." For example, in the experiment reported in table 2 the 1st, 6th and 11th bottles filled were chosen as blanks. The average of the analyses of the blanks was taken to represent the amount of oxygen in each of the other bottles at the beginning of the experiment. Uniformity among blanks, chosen in this way, was taken to show uniformity of filling of the whole series of experimental bottles.

Worms were introduced into experimental bottles by means of a glass pipette of suitable size. With care, a worm can be introduced into a bottle by touching the pipette to the surface of the water in the bottle without introducing more than a drop of water. In removing worms at the end of an experiment, the Planaria agilis were loosened by gently rotating the bottle, and were then picked out individually by means of the pipette. In this operation, care was taken not to introduce any bubbles of air in the bottle and not to draw into the pipette any water from the vessel into which the worms were transferred. Planaria maculata cling to the substratum very tenaciously. Frequently it was necessary to loosen them by means of a wire probe, flattened at the end to form a spatula. The following tests show that the error from introducing probes and pipettes into bottles to remove worms was negligible. Seven bottles were filled with aerated tap water. Four of these were analyzed as controls, giving an average oxygen content of 4.03 cc. thiosulphate. Each of the other three bottles was opened and the pipette was introduced twenty-five times, drawing in and expelling water thrice each time. The average oxygen content of these bottles was then found to be 4.04 cc. thiosulphate, showing no difference from the controls. The water in the bottles in this case was approximately in equilibrium with air as to its dissolved oxygen. Under such circumstances it was not to be expected that the manipulations described would affect the oxygen content appreciably.

At the end of an experiment, however, after part of the oxygen has been consumed, the water is no longer in equilibrium with air and these manipulations might alter the oxygen content. The following test meets these conditions. Six bottles were filled with boiled tap water of low oxygen content. Three alternate bottles were analyzed as controls, giving an average oxygen content of 1.74 cc. thiosulphate. Into each of the other three bottles ten worms were introduced and then immediately removed. These experimental bottles gave an average oxygen content of 1.80 cc. thiosulphate, showing a difference of 0.06 cc. thiosulphate from the controls. In the experiments to be reported, the oxygen content of the bottles at the end of the experimental periods was rarely reduced below 2.50 cc. thiosulphate. Moreover, any error from this source would tend to diminish differences in oxygen consumption rather than to produce them to any degree.

In some cases, removing worms removed so much water that there was danger of enclosing a bubble of air in closing the bottle after introducing the reagents. To prevent this possibility, the uniform procedure was followed of displacing 2 cc. of water with a glass rod before introducing the reagents. The rods used for this purpose were of the same volume and were marked so that they could not be confused with other rods present in certain bottles for the purpose of standardizing the volume. This procedure was adopted in experiments with Planaria maculata, which are difficult to handle with a pipette, and was continued in a routine manner in all experiments. In all cases blanks were handled in the same manner as experimental bottles in this respect. In calculating the oxygen consumption in cubic centimeters of oxygen gas, cf. table 10, columns 3 and 6, correction was made for the 4 cc. displaced by the glass rods as well as for the 2 cc. displaced by the added reagents. These corrections were not made in the tables giving the oxygen consumption in cubic centimeters of thiosulphate since they do not alter relative values.

It required from two to four minutes to remove worms from a bottle and introduce the reagents. When an experiment contained several parts and there were two or three duplicate bottles in each part, it often required half an hour or even an hour to carry out these manipulations. Under such circumstances, to avoid serious error from the fact that the length of the experimental period was greater for certain bottles than for others, bottles were handled in the same order at the end of the period as at the beginning. In all experiments a record was made of the exact time of handling each bottle, and these times were so arranged as to insure that there was no variation from bottle to bottle in the length of the experimental period.

The following test shows that the iodine error from mucous secretions of the worms was also negligible. In each of four bottles, six worms were left for four and a half days, after which time they were removed and the bottles were filled slowly with aerated tap water so as to avoid violent currents that might remove accumulations of mucus. The average of the four analyses was 4.63 cc. thiosulphate, while four clean bottles filled at the same time from the same aerated tap water gave an average analysis of 4.65 cc. thiosulphate.

In the experiments reported in the present paper, each contains its own temperature control since all comparisons that have been made are between worms that were tested simultaneously and under the same conditions of temperature. However, except in the first two preliminary experiments, all bottles were placed in a water bath within a constant temperature oven at 20°C., so that different experiments are comparable so far as temperature is concerned.

For each experiment the potassium cyanide was freshly weighed out, and a relatively strong solution, e.g., 0.01 molecular, was made with the aerated tap water used in the experiment. The bottles were filled in the usual manner. Those bottles that were not to contain cyanide were closed at once. A measured amount of the stronger cyanide solution was run in from a pipette or burette at the bottom of the other bottles, which were then closed and shaken well before introducing worms. The concentration of the solution was regulated so that the amount of solution added never exceeded 5 cc. and was usually about 2 cc. Upon opening the bottles, before introducing worms, a 2 cc. rod was inserted into each, displacing enough water to make it certain that it could be closed again without leaving any bubbles of air. All bottles in the experiment, including the blanks, were handled alike in this respect.

Potassium cyanide in the concentrations used does not cause any appreciable error in the Winkler oxygen analysis. This was shown by a preliminary test in which three bottles of aerated tap water containing KCN in 0.0004 molecular concentration gave an average oxygen analysis of 4.00 cc. thiosulphate, while three bottles of aerated tap water without cyanide gave an average of 3.99 cc. thiosulphate. In some experiments cyanide solution was introduced into part of the blanks but no appreciable difference in the analyses was found between them and the other blanks not containing KCN, so that this procedure was not always followed.

The experimental method has been explained in considerable detail because several sources of error might readily occur if care is not taken in the details of manipulation. However, when careful methods are followed, their accuracy may be judged by their results. When duplicate determinations in the same experiment show uniformity of result and when a series of determinations with graduated differences in a certain experimental factor such as concentration of KCN shows a consistently graduated series of differences in result, the experiment carries conviction of reliability even when the actual figures are not large. All experiments have been carried out in duplicate of two or three simultaneous but independent determinations as a control upon the accuracy of the methods. The tables show all the individual determinations, even at the expense of making the tables more involved, in order that the uniformity among duplicate determinations may be clearly seen.

OXYGEN CONSUMPTION BY PLANARIA IN KCN SOLUTIONS OF DIFFERENT CONCENTRATIONS

To determine, first of all, whether KCN decreases the oxidations in Planaria, a preliminary experiment was performed with Planaria maculata, in which the oxygen consumption was tested first in aerated tap water and next in aerated tap water containing KCN. Table 2 states the conditions and gives the results. The headings of the columns are self explanatory. Part A gives the analyses of the blanks, which tell the amount of oxygen in the experimental bottles of part B at the beginning of the experiment, and which serve also as a control for the uniformity of filling of the experimental bottles. The first test in aerated tap water is a control to show the degree of uniformity of oxygen consumption by the animals used in the different bottles of the experiment. In the second test, the bottles of set I contained no KCN, and serve, therefore, as a control for those sets in which cyanide was used. Bottles of set II contained the strongest KCN solution, set III a weaker solution and set IV the most dilute solution. Column 4 shows that in every case worms in cyanide solutions used much less oxygen than those in pure water. In the previous test, column 2, they all used approximately the same amounts of oxygen. Column 4 indicates also that worms in the weakest solutions, i.e., set IV, used more oxygen than those in stronger solutions, but the worms in the stronger solutions suffered injury, as is shown in column 6, so that this experiment is inconclusive so far as concerns the relative effects of KCN solutions of different concentrations. Worms of set IV, however, all of which remained alive and uninjured in 0.000076 molecular KCN, showed a reduction of over half in their oxygen consumption.

A second preliminary experiment was then performed with Planaria agilis, using weaker solutions of KCN. The conditions and results are shown in table 3. The general organization of the experiment and the controls was the same as in the former experiment. Column 2 shows again that worms in cyanide solutions invariably consumed less oxygen than those in pure water, while column 1 shows that in the preliminary test in pure water they all consumed approximately the

TABLE 2

Preliminary experiment showing decrease in the oxygen consumption by Planaria maculata in KCN solutions. Worms, medium size, in laboratory six days without food, 10 individuals in each bottle. Room temperature 20° to 24°C. 1 cc. thiosulphate = 0.1751 cc. oxygen at N. T. P.

PART	SET	BOTTLE	FIRST TEST (CONTROL) (DURATION 12.13 HOURS)			SECOND TEST (DURATION 10.5 HOURS)				
			Blanks			Blanks				
			cc. thio.			*cc. thio.*				
A	Blanks (no worms present)	1	3.36			3.90				
		2	3.32			3.95				
		3	3.33			3.91				
	Average		3.34			3.92				
			(1)	(2)		(3)	(4)		(5)	(6)
			Oxygen remaining at end of period	Oxygen consumed		Oxygen remaining at end of period	Oxygen consumed		Concentration of KCN in each bottle	Condition of worms at end of test
			cc. thio.	*cc. thio.*	*average*	*cc. thio.*	*cc. thio.*	*average*		
B	I	1	2.69	0.68	0.67	3.33	0.62	0.64	No KCN	All alive
		2	2.65	0.69		3.29	0.63			
		3	2.70	0.64		3.23	0.66			
	II	1	2.64	0.70	0.70	3.73	0.19	0.19	0.00015 M	3 disintegrating 2 curled up
		2				3.74	0.18			2 disintegrating 4 curled up
	III	1	2.69	0.65	0.67	3.80	0.12	0.15	0.000114 M	4 disintegrating 1 curled up
		2	2.65	0.69		3.74	0.18			4 disintegrating 2 curled up
	IV	1	2.78	0.56	0.61	3.70	0.22	0.24	0.000076 M	All alive
		2	2.69	0.65		3.66	0.26			

same amounts. In this experiment all worms remained alive and uninjured. A comparison of the averages in columns 2 and 4 shows that there is an inverse relation between the concentration of the cyanide and the oxygen consumption or, in other words, that there is a direct relation between the concentration of KCN and the decrease in the oxygen consumption. The averages of column 2 have been converted into percentages in column 3, where it is seen that the oxygen consumption in 0.000076 molecular KCN was only 38 per cent of

TABLE 3

Preliminary experiment showing decrease in the oxygen consumption by Planaria agilis in KCN solutions. Worms, 11 to 13 mm. in length; fed beef liver daily till three days previous to the experiment; 10 individuals in each bottle. Each test period of 8 hours duration. Room temperature, 22° to 24°C. 1 cc. thiosulphate = 0.1751 cc. oxygen at N. T. P.

PART	SET	BOTTLE	FIRST TEST (CONTROL)		SECOND TEST				
			Blanks		Blanks				
			cc. thio.	*average*	*cc. thio.*	*average*			
A	Blanks (no worms present)	1	3.80	*3.79*	3.74	*3.73*	No KCN		
		2	3.80		3.69		No KCN		
		3	3.81		3.75		0.000038 Mol. KCN		
		4	3.76		3.73		0.000038 Mol. KCN		
			(1)		(2)		(3)	(4)	(5)
			Oxygen consumed (Control)		Oxygen consumed		Average rate of oxygen consumption in per cent of normal	Concentration of KCN in each bottle	Condition of worms at end of test
			cc. thio.	*average*	*cc. thio.*	*average*	*per cent*		
B	I	1	0.52	*0.52*	0.38	*0.40*	(Normal)	No KCN	All alive
		2	0.51		0.42				
	II	1	0.47	*0.49*	0.14	*0.15*	*38*	0.000076M	All alive
		2	0.50		0.16				
	III	1	0.56	*0.51*	0.27	*0.24*	*60*	0.000038M	All alive
		2	0.45		0.21				
	IV	1	0.52	*0.52*	0.35	*0.35*	*88*	0.0000076M	All alive
		2	0.52		0.35				

normal. In the previous experiment, table 2, the oxygen consumption in this same concentration of cyanide was 36 per cent of normal.

It might be noted that at the end of this experiment, when the worms in cyanide were disturbed, they all became active but there was a very marked gradation in the rate of locomotion in the different concentrations of KCN. In the strongest cyanide the rate was least while in the weakest cyanide the rate was only slightly less than that of the worms in pure water.

In order to determine the general character of the proportionality between the concentration of the cyanide and the amount of oxygen consumed, the experiment summarized in table 4 was performed. The general plan of the experiment was the same as that of the previous experiments except that a greater number and a wider range of concentrations of KCN were used. Seven different concentrations were employed, ranging from 0.00000076 molecular to 0.00038 molecular. Column 1 shows a high degree of uniformity in the oxygen consumption of the different sets in the first test without cyanide. Column 2 shows that in the second test the oxygen consumption decreased very regularly as the concentration of KCN increased. In the strongest solution, set VIII, the worms were all disintegrated at the end of the experimental period so that the figures in this part of the table may, perhaps, be neglected. Worms in sets VI and VII seemed to be near the limit of their resistance and the figures of set VII might be questioned.

While the experiment recorded in table 4 gave very consistent results, the absolute values were so small that the experiment was repeated with larger worms, as summarized in table 5. The "second part" of this experiment will be discussed under the topic "Reversibility of the action of KCN on Planaria." For the present, attention is confined to the "first part," i.e., to the first and second tests. In this experiment all worms remained alive and uninjured. In the control test, the worms in each of the different bottles consumed approximately the same amount of oxygen, column 1. In the cyanide test the worms in 0.00023 molecular KCN, set VII, consumed only 28 per cent as much oxygen as the control, set I. Worms in 0.00015 molecular KCN consumed 35 per cent as much as the control and worms in 0.000076 molecular KCN consumed 53 per cent as much oxygen as the control. In the still weaker concentrations the oxygen consumption was practically the same as the control. These concentrations were very dilute and were chosen to see whether minimal

TABLE 4

Showing the relation of decrease in the oxygen consumption by Planaria agilis in KCN solutions to the concentration of the KCN. Worms, 10 to 13 mm. in length; fed beef liver almost daily till 12 days previous to experiment; 10 individuals in each bottle. Temperature 19.7° to 19.8°C. 1 cc. thiosulphate = 0.1751 cc. oxygen at N. T. P.

PART	SET	BOTTLE	FIRST TEST (CONTROL) (DURATION 12 HOURS)		SECOND TEST (DURATION 15 HOURS)				
			Blanks		Blanks				
			cc. thio.	*average*	*cc. thio.*			*average*	
A	Blanks (no worms present)	1	4.05	*4.06*	4.17			*4.17*	
		2	4.08		4.17				
		3	4.07		4.17				
		4	4.04		4.15				
			(1)		(2)		(3)	(4)	(5)
			Oxygen consumed (Control)		Oxygen consumed		Average rate of oxygen consumption in per cent of normal	Concentration of KCN in each bottle	Condition of worms at end of test
			cc. thio.	*average*	*cc. thio.*	*average*	*per cent*		
B	I	1	0.30	*0.29*	0.39	*0.39*	(Normal)	No KCN	All alive
		2	0.27		0.39				
	II	1	0.30	*0.32*	0.36	*0.36*	*92*	0.00000076M	All alive
		2	0.33		0.36				
	III	1	0.33	*0.32*	0.38	*0.35*	*90*	0.0000076M	All alive
		2	0.30		0.32				
	IV	1	0.32	*0.32*	0.25	*0.24*	*62*	0.000038M	All alive
		2	0.32		0.22				
	V	1	0.32	*0.34*	0.18	*0.20*	*51*	0.000076M	All alive
		2	0.35		0.21				
	VI	1	0.33	*0.30*	0.15	*0.15*	*38*	0.000114M	Slightly injured
		2	0.27		0.15				Slightly injured
	VII	1	0.35	*0.33*	0.17	*0.13*	*33*	0.00015M	Injured
		2	0.31		0.09				Injured
	VIII	1	0.33	*0.31*	0.11	*0.09*	*23*	0.00038M	All disintegrated
		2	0.28		0.06				All disintegrated

TABLE 5

First part (first and second tests) showing the relation of the decrease in the oxygen consumption by Planaria agilis in KCN solutions to the concentration of KCN. Worms, 15 to 16 mm. in length and very broad, without food for 28 days previous to experiments, 10 individuals in each bottle. Each test period of 12 hours duration, with 12 hours interval between tests. Temperature 19.5° to 20.0°C. 1 cc. thiosulphate = 0.1729 cc. oxygen at N. T. P.

Second part (third test) showing reversibility of the effect of KCN in reducing the oxygen consumption. Third test follows second test after interval of 12 hours.

PART	SET	BOTTLE	FIRST PART				SECOND PART	
			First test (Control)		Second test		Third test	
			Blanks		Blanks		Blanks	
			cc. thio.	*average*	*cc. thio.*	*average*	*cc. thio.*	*average*
	Blanks (no worms present)	1	4.39	*4.40*	4.38	*4.36*	4.34	*4.31*
		2	4.41		4.38		4.31	
		3	4.39		4.33		4.29	
		4	4.40		4.36		4.31	

PART	SET	BOTTLE	(1)		(2)		(3)	(5)	(6)	
			Oxygen consumed (Control)		Oxygen consumed		Average rate of oxygen consumption in per cent of normal	Concentration of KCN in each bottle	Oxygen consumed	
			cc. thio.	*average*	*cc. thio.*	*average*	*per cent*		*cc. thio.*	*average*
B	I	1	1.00	*1.02*	0.99	*0.97*	(Normal)	No KCN	1.04	*1.00*
		2	1.03		0.93				0.96	
		3	1.02		0.99				0.99	
	II	1	1.10	*1.07*	1.01	*1.01*	*104*	0.00000015M	1.03	*1.00*
		2	1.10		1.05				1.01	
		3	1.02		0.98				0.96	
	III	1	1.05	*1.03*	1.01	*1.00*	*103*	0.00000076M	1.01	*0.98*
		2	1.03		0.99				0.98	
		3	1.00		1.00				0.95	
	IV	1	1.05	*1.05*	0.94	*0.98*	*101*	0.0000076M	1.01	*1.03*
		2	1.01		0.96				1.01	
		3	1.08		1.04				1.06	
	V	1	0.90	*0.98*	0.51	*0.51*	*53*	0.000076M	0.96	*0.99*
		2	1.03		0.50				1.01	
		3	1.02		0.53				1.01	
	VI	1	1.04	*1.04*	0.32	*0.34*	*35*	0.00015M	1.01	*1.06*
		2	1.11		0.36				1.14	
		3	0.97		0.34				1.02	
	VII	1	1.13	*1.03*	0.29	*0.27*	*28*	0.00023M	1.15	
		2	1.04		0.28				1.	
		3	0.92		0.25				1.	

quantities of cyanide cause marked decrease or increase in the oxygen consumption. The results showed neither effect. The percentages in column 3 run slightly above the control but the difference is not significant, especially when it is considered that sets II, III and IV used more oxygen than set I in the control test also, column 1. This point has not been tested further as yet, but so far as these experiments go they do not give any evidence of a "stimulating effect" of very weak KCN upon the oxygen consumption of Planaria.

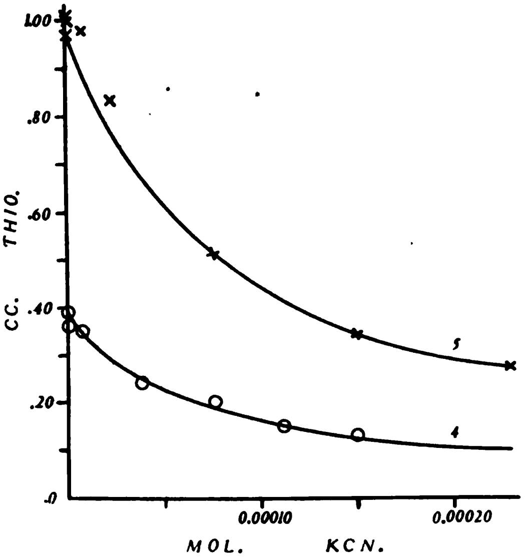

Fig. 1. Curves showing decrease in the oxygen consumption by Planaria agilis in KCN solutions of different concentration. Abscissas = molecular concentration of KCN. Ordinates = average oxygen consumption per bottle of worms, in cubic centimeters of thiosulphate. Curve *4* represents the data of table 4, column 2. Curve *5* represents the data of table 5, column 2.

The data of tables 4 and 5 have been plotted in figures 1 and 2. Figure 1 shows the average oxygen consumption per bottle in cubic centimeters of thiosulphate, plotted against the concentration of KCN. The curve marked *4* represents the data from table 4, and the curve marked *5* represents the data from table 5. Each point on curve *4* represents, therefore, the average of two determinations, and each point on curve *5* represents the average of three determinations. The two curves do not coincide because they represent the absolute amounts of oxygen consumed by worms of different sizes, but it can be seen at a glance that they have the same general form. Figure 2 shows the average oxygen consumption in different KCN solutions, expressed in per cent of the control. As in figure 1, the curve marked *4* represents the data of table 4 and the curve marked *5* represents the data of table 5. Four things may be noted in figure 2: first, that the two curves almost coincide, showing that the two experiments, tables 4 and 5, give identical results; second, that the curves fall sharply; third, that they are not straight lines; and fourth, that they approach the horizontal at about 20 per cent of the normal. We may conclude, therefore, that lower concentrations of KCN have proportionately greater effect upon the oxidations than more concentrated solutions; and second, that the oxidations cannot be reduced by cyanide below about 20 per cent of the normal. In other words, these results indicate that a residual oxidation amounting to about 20 per cent of the normal cannot be inhibited by KCN. No clear evidence is obtained from experiments 4 and 5 for a stimulation of oxidations in weak solutions of KCN.

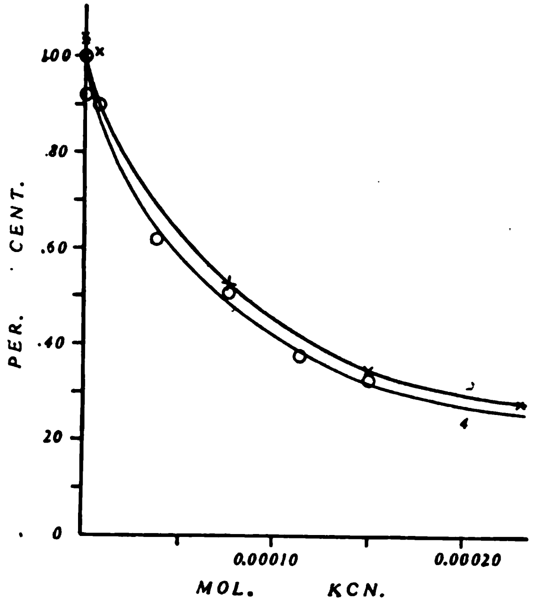

Fig. 2. Curves showing decrease in the rate of oxygen consumption by Planaria agilis in KCN solutions of different concentration. Abscissas = molecular concentration of KCN. Ordinates = rate of oxygen consumption in per cent of normal. Curve *4* represents the data of table 4, column 3. Curve *5* represents the data of table 5, column 3.

OXYGEN CONSUMPTION BY PLANARIA IN KCN SOLUTIONS DURING TIME PERIODS OF DIFFERENT LENGTH

It is possible that the rate at which Planaria use oxygen in KCN solutions changes with time. Miss Hyman (7) reports finding such changes in the oxygen consumption by sponges for the first two or three half-hour periods. But when longer experimental periods are employed, as in the experiments reported in this paper, it seems improbable that the precise length of the period should alter the general character of the results. However, to make certain regarding this point, the following experiment was performed. Different sets of worms were tested for periods of different length from 2 to 36 hours. All sets were started at the same time. Set I was stopped after 2 hours, set II after 4 hours, set III after 8 hours, etc., as can be seen in column 1, table 6. Each set comprised two bottles of worms used as a control set and two bottles used as an experimental set.

All sets were tested first in aerated tap water as a general control for the second test, in which the experimental sets were treated with KCN. This preliminary control test was carried out in every respect like the second test except that no KCN was present in any of the bottles. Attention may be called to several points in the results of the control test which give evidence regarding the accuracy of the methods. It will be noted in column 2 that for the short periods more worms were used in each bottle and for the longer periods fewer individuals were used. Column 3, which states the amount of oxygen absorbed by the worms in each bottle in cubic centimeters of thiosulphate, shows that not more than the equivalent of 2.20 cc. thiosulphate was absorbed by the worms in any bottle, i.e., not more than about half of the amount of oxygen available in each bottle at the beginning of the period, 4.21 cc. thiosulphate. The worms of the longer experimental periods were not subjected, therefore, to oxygen starvation. In the next place, the degree of uniformity among the individual determinations is shown in column 4. In this column the oxygen consumption has been reduced to the common basis of the number of cubic centimeters of oxygen gas at normal temperature and pressure absorbed per 10 worms per 24 hours. The individual determinations ranged from 4.02 to 4.50 cc. of oxygen, with the exception of one determination, set Vb, bottle 1, which was as low as 3.38 cc. oxygen. An examination of further records of this particular lot of worms is instructive. Table 8 gives the oxygen consumption by the worms in each

TABLE 6

Showing that Planaria agilis in a KCN solution maintains approximately the same rate of oxygen consumption during time periods of very different length. Worms 15 to 16 mm. in length and very broad; without food for 18 days previous to the experiment except feedings on the 5th and 6th days before the experiment. Concentration of KCN = 0.00003 molecular. All bottles started at the same time and analyzed after periods of different length, as noted. Temperature 1.94° to 20 0°C. All worms alive at the end of the experiment. 1 cc. thiosulphate = 0.1747 cc. oxygen at N. T. P.

PART	SET		BOTTLE			FIRST TEST (CONTROL)				SECOND TEST			
						Blanks				Blanks			
						cc. thio	*average*			*cc. thio*	*average*		
A	Blanks (No worms present)		1			4.18	*4.21*			4.05	*4.08*		No KCN
			2			4.24				4.10			No KCN
			3			4.23				4.10			KCN
			4			4.18				4.08			KCN
				(1) Length of test period	(2) Number of worms in each bottle	(3) Oxygen consumed (Control)		(4) Oxygen consumption per 10 worms per 24 hours	(5) Average rate of oxygen consumption in per cent of normal	(6) Oxygen consumed		(7) Average rate of oxygen consumption in per cent of normal	(8) KCN or no KCN
				hrs.		*cc. thio*	*aver-age*	*cc. oxygen*	*per cent*	*cc. thio*	*aver-age*	*per cent*	
B	I	a	1	2	20	0.72	*0.73*	4.32	(Normal)	0.71	*0.72*	(Normal)	No KCN
			2	2	20	0.74		4.44		0.73			No KCN
		b	1	2	20	0.71	*0.73*	4.26	*100*	0.53	*0.47*	*65*	KCN
			2	2	20	0.75		4.50		0.41			
	II	a	1	4	20	1.42	*1.38*	4.26	(Normal)	1.28	*1.29*	(Normal)	No KCN
			2	4	20	1.34		4.02		1.30			
		b	1	4	20	1.37	*1.42*	4.11	*103*	0.84	*0.84*	*65*	KCN
			2	4	20	1.46		4.38		0.84			
	III	a	1	8	15	2.11	*2.11*	4.22	(Normal)	2.02	*2.01*	(Normal)	No KCN
			2	8	15	2.10		4.20		1.99			
		b	1	8	15	2.13	*2.09*	4.26	*99*	1.20	*1.17*	*58*	KCN
			2	8	15	2.05		4.10		1.13			

TABLE 6—*Continued*

PART	SET		BOTTLE	(1)	(2)	(3)		(4)	(5)	(6)		(7)	(8)
				Length of test period	Number of worms in each bottle	Oxygen consumed (Control)		Oxygen consumption per 10 worms per 24 hours	Average rate of oxygen consumption in per cent of normal	Oxygen consumed		Average rate of oxygen consumption in per cent of normal	KCN or no KCN
				hrs.		*cc. thio*	*average*	*cc. oxygen*	*per cent*	*cc. thio*	*average*	*per cent*	
B	IV	a	1	12	10	2.06	*2.08*	4.12	(Normal)	1.98	*2.05*	(Normal)	No KCN
			2	12	10	2.10		4.20		2.11			
		b	1	12	10	2.03	*2.06*	4.06	*99*	1.10	*1.12*	*55*	KCN
			2	12	10	2.08		4.16		1.13			
	V	a	1	24	5	2.20	*2.18*	4.40	(Normal)	2.08	*2.07*	(Normal)	No KCN
			2	24	5	2.15		4.30		2.05			
		b	1	24	5	1.69	*1.87*	3.38	*86*	0.92	*1.02*	*49**	KCN
			2	24	5	2.04		4.08		1.12			
	VI	a	1	36	3	1.88	*1.95*	4.16	(Normal)	1.73	*1.81*	(Normal)	No KCN
			2	36	3	2.02		4.49		1.88			
		b	1	36	3	1.97	*2.00*	4.38	*103*	1.08	*1.09*	*60*	KCN
			2	36	3	2.02		4.49		1.10			

* Corrected to 57 per cent, cf. text page 111.

bottle of sets Va and Vb for five successive tests. In every test this particular lot of worms, set Vb 1, absorbed much less oxygen than any other lot. Evidently this lot of worms was not equivalent to the others in its normal capacity for absorbing oxygen and the low value of 3.38 cc. in table 6, column 4, set Vb 1, was due to irregularity of material rather than to any error in experimental measurement. Part of the variation between the other determinations also may be due to irregularities of material since the worms were selected for uniformity of size without measurement of their actual weights. It may be noted that column 4, table 6, shows no appreciable difference between the normal rate of oxygen absorption during short and longer periods of time. This is further evidence of the reliability of the experimental methods.

The amount of oxygen absorbed by the worms in each bottle during the second test is given in cubic centimeters of thiosulphate in column 6. In this test the experimental worms were treated with 0.00003 molecular KCN. This concentration was chosen because it has been found to cause a reduction in oxygen consumption which is intermediate between the maximum and minimum effects of cyanide. The oxygen consumption by worms in this solution during each of the different periods of time, from two to thirty-six hours, was compared with that of the corresponding untreated worms. For example, the average oxygen consumption by the control set, Ia, which were in pure water for 2 hours, was 0.72 cc. thiosulphate, column 6. The average oxygen consumption by the experimental set, Ib, which were in cyanide for the same period of 2 hours, was 0.47 cc. thiosulphate or 65 per cent of the normal. Similarly the average oxygen consumption of the other control sets: IIa, IIIa, IVa, Va, and VIa, column 6, were each taken as normal or 100 per cent, column 7, with which were compared the average oxygen consumption of the corresponding experimental sets: IIb, IIIb, IVb, Vb, and VIb. Similar calculations for the first test, column 5, showed that the experimental sets were equivalent to the control sets in every case excepting set V, which has been discussed above. Column 7 shows, then, that the cyanide-treated worms in the second test used about 60 per cent of the normal amount of oxygen. As explained above, the worms in set Vb 1 were not equivalent to those in other bottles. When this fact is taken into account, the 49 per cent in column 7, set Vb, becomes 57 per cent.

In this experiment the rate of oxygen consumption in cyanide solution was practically the same during periods of different length from two to thirty-six hours. The inhibitory action of KCN on oxidations in Planaria was immediate or very rapid, therefore, and it did not change with time, at least within the limits of the experiment. There was no evidence of an initial stimulating effect of cyanide on the oxidations.

REVERSIBILITY OF THE ACTION OF KCN ON PLANARIA

Potassium cyanide is sometimes classed among the anesthetics. One of the characteristics of the action of the anesthetics is their reversibility, i.e., the fact that if the anesthetic has not been given in too strong concentration and has not acted for too long a time, the organism recovers from its effect rapidly and completely after removal from its action. Several experiments demonstrated that the action of KCN on the oxygen absorption by Planaria is reversible.

Table 5, column 5, shows the complete recovery of the original rate of oxidation by Planaria after their removal from solutions of cyanide in which their oxidations were only 28 to 53 per cent of the normal.

TABLE 7

Showing reversibility of the effect of KCN on the oxygen consumption by Planaria agilis. Worms 15 to 16 mm. in length and very broad; without food for 18 days preceding the experiment except feedings on the 5th and 6th days previous to experiment; 20 individuals in each bottle. Each test period of 4 hours duration. Concentration of KCN solution = 0.00003 molecular. After worms were removed from the KCN of the 2nd test they were washed repeatedly in tap water and set for the 3rd test within 30 minutes. Data of the 1st and 2nd tests included also in table 6. Temperature 19.4° to 20.0°C. 1 cc. thiosulphate = 0.1747 cc. oxygen at N. T. P.

PART	SET	BOTTLE	FIRST TEST (CONTROL)		SECOND TEST			THIRD TEST		FOURTH TEST	
			Blanks		Blanks			Blanks		Blanks	
			cc. thio	*average*	*cc. thio*	*average*		*cc. thio*	*average*	*cc. thio*	*average*
A	Blanks (No worms present)	1	4.18	*4.21*	4.05	*4.08*	No KCN	4.31	*4.34*	4.36	*4.37*
		2	4.24		4.10		No KCN	4.34		4.39	
		3	4.23		4.10		KCN	4.38		4.40	
		4	4.18		4.08		KCN	4.32		4.34	
			(1)		(2)		(3)	(4)		(5)	
			Oxygen consumed		Oxygen consumed		KCN or no KCN	Oxygen consumed		Oxygen consumed	
			cc. thio	*average*	*cc. thio*	*average*		*cc. thio*	*average*	*cc. thio*	*average*
B	IIa	1	1.42	*1.38*	1.28	*1.29*	No KCN	1.30	*1.34*	1.40	*1.43*
		2	1.34		1.30			1.38		1.45	
	IIb	1	1.37	*1.42*	0.84	*0.84*	KCN	1.41	*1.43*	1.48	*1.47*
		2	1.46		0.84			1.44		1.46	
Per cent of normal				*103*		*65*			*107*		*103*

Twelve hours intervened between the second and third tests in this experiment so that the experiment gives no idea of the rapidity of the recovery of the normal rate, but it does show completeness of recovery.

Table 7 shows that the recovery is as rapid as is the action of the cyanide in reducing the oxidations. The first test, column 1, was the control test. The second test, column 2, showed a decrease in the oxygen consumption to 65 per cent of the normal in 0.00003 molecular KCN. The third and fourth tests show complete recovery of the normal consumption in tap water. The third test followed the second with only thirty minutes interval. In this experiment the test periods were each of four hours' duration. The first and second tests of this table are included also in table 6. After a short exposure, therefore, to cyanide which reduced the oxygen consumption 40 per cent, the consumption rose again to normal after removal from the cyanide solution, as rapidly as it fell in the cyanide.

TABLE 8

Showing reversibility of the effect of KCN on the oxygen consumption by Planaria agilis. Worms 15 to 16 mm. in length and very broad; without food for 18 days preceding experiment except for feedings on the 5th and 6th days previous to the experiment; 5 individuals in each bottle. Concentration of KCN, 0.00003 molecular. After worms were removed from KCN of the 2nd test they were washed repeatedly in tap water and put into bottles for the 3rd test within 17 minutes. Temperature 19.4 to 20.0°C. 1 cc. thiosulphate = 0.1747 cc. oxygen at N. T. P. Data of 1st and 2nd tests included in table 6.

PART	SET	BOTTLE	FIRST TEST		SECOND TEST			THIRD TEST		FOURTH TEST		FIFTH TEST	
			Blanks		Blanks			Blanks		Blanks		Blanks	
			cc. thio	*average*	*cc. thio*	*average*	*KCN or no KCN*	*cc. thio*	*average*	*cc. thio*	*average*	*cc. thio*	*average*
A	Blanks (No worms present)	1	4.18	*4.21*	4.05	*4.08*	No KCN	4.23	*4.27*	4.35	*4.36*	4.17	*4.18*
		2	4.24		4.10		No KCN	4.30		4.38		4.18	
		3	4.23		4.10		KCN	4.31		4.39		4.20	
		4	4.18		4.08		KCN	4.24		4.32		4.16	
			(1)		(2)		(3)	(4)		(5)		(6)	
			Oxygen consumed in 24 hours		Oxygen consumed in 24 hours		KCN or no KCN	Oxygen consumed in 6 hours		Oxygen consumed in 6 hours		Oxygen consumed in 24.6 hours	
			cc. thio	*average*	*cc. thio*	*average*		*cc. thio*	*average*	*cc. thio*	*average*	*cc. thio*	*average*
B	Va	1	2.20	*2.18*	2.08	*2.07*	No KCN	0.63	*0.64*	0.59	*0.59*	2.00	*1.98*
		2	2.15		2.05			0.65		0.58		1.95	
	Vb	1	1.69	*1.87*	0.92	*1.02*	KCN	0.52	*0.60*	0.49	*0.53*	1.52	*1.71*
		2	2.04		1.12			0.67		0.56		1.89	
Per cent of control				*86*		*49*			*94*		*90*		*86*

Table 8 shows a similar rapid recovery of the normal oxygen consumption by worms after long exposure to cyanide solutions of the same concentration. In the control test, test 1, set Vb, the experimental worms used 86 per cent as much oxygen as the control worms, test 1, set Va, so that this is the normal consumption of these worms. In the second test the worms in 0.00003 molecular KCN, column 2, set Vb, used 49 per cent as much oxygen as the control, column 2, set Va, i.e., they used 57 per cent as much as was normal for the experimental worms. In the third, fourth and fifth tests, the experimental worms recovered their normal consumption of oxygen. The first and second tests were each twenty-four hours in duration and the third and fourth were each six hours in duration. The interval between the second and third tests was only seventeen minutes. After exposure to the action of cyanide for twenty-four hours, therefore, the worms recovered so rapidly after removal from the cyanide solution that the normal rate was maintained for the first six hours. The first and second tests of this experiment are included also in table 6.

RELATION BETWEEN THE OXYGEN CONSUMPTION BY PLANARIA IN KCN AND LOCOMOTOR ACTIVITY

Since it has been demonstrated by numerous experiments that the oxygen consumption by Planaria falls very markedly in KCN solutions, the question arises whether this decrease can be accounted for by decreased locomotion in the cyanide solutions. The locomotion of Planaria can be stopped, as has been stated by Child (1, p. 161), by cutting off the head and this operation does not involve serious injury to the worm. To get a picture of how effective this measure is in stopping locomotion, the experiment recorded in table 9 was performed. Four bottles containing five worms each were placed in a darkened room where they could be observed very carefully without disturbing the worms. The first bottle contained normal worms; the second bottle, worms with the heads removed close behind the auricles; the third bottle, worms with the heads removed a short distance in front of the pharynx; and the fourth bottle contained worms in a 0.000076 molecular solution of KCN. All bottles were observed at frequent intervals during eight hours. At each observation the number of worms found in movement in each bottle was recorded. After the first few observations the positions of the different individuals were easily remembered and any worm which had changed position since the last observation was counted as in motion. This was very convenient in the case of the worms in the cyanide since they moved very slowly. The table shows that the removal of the head stops movement immediately and almost completely. After the original disturbance the normal worms required some time before coming to rest, but once at rest they remained so for hours, if not disturbed. The bottle of cyanide-treated worms was started thirty-five minutes later than the other bottles, but if the table is read with this is mind it will be seen that the worms in cyanide were in motion for even more of the time than the normal worms not in cyanide, although the rate of motion in cyanide was very slow. This experiment shows, there-

TABLE 9

Showing that removal of the head of Planaria agilis is more effective than 0.000076 molecular KCN in stopping locomotion. Worms 12 to 14 mm. in length; 5 individuals in each bottle. Worms of bottle I were normal. Bottle II, heads of worms removed a short distance posterior to auricles at 9:00 a.m. Bottle III, heads removed a short distance anterior to pharynx. Bottles I, II and III placed in dim light at 9:05 a.m. and not again disturbed. Bottle IV, worms placed in 0.000076 molecular KCN at 9:40 a.m.

A NUMBER OF INDIVIDUALS IN MOTION OR MOVED SINCE LAST OBSERVATION					A—*continued*					A—*continued*				
	I	II	III	IV		I	II	III	IV		I	II	III	IV
	Normal	Headless	Headless	KCN		Normal	Headless	Headless	KCN		Normal	Headless	Headless	KCN
9:10 a.m.	5	0	1		10:38 a.m.	2	0	0	4	1:30 p.m.	0	0	0	3
9:15 a.m.	5	0	0		10:45 a.m.	1	0	0	2	1:45 p.m.	0	0	0	3
9:20 a.m.	5	0	0		10:50 a.m.	1	0	0	2	2:05 p.m.	0	0	0	1
9:25 a.m.	4	0	0		10:55 a.m.	1	0	0	2	2:15 p.m.	0	0	0	1
9:30 a.m.	4	0	0		11:00 a.m.	2	0	0	3	2:30 p.m.	0	0	0	0
9:35 a.m.	3	0	0		11:05 a.m.	1	0	0	2	2:45 p.m.	0	0	0	1
9:40 a.m.	3	0	1		11:10 a.m.	1	0	0	2	3:00 p.m.	0	0	0	1
9:45 a.m.	3	0	0	5	11:15 a.m.	1	0	0	1	3:20 p.m.	0	0	0	1
9:50 a.m.	3	0	1	5	11:20 a.m.	1	0	0	2	3:45 p.m.	0	0	0	0
9:55 a.m.	4	0	1	5	11:25 a.m.	1	0	0	3	4:00 p.m.	0	0	0	0
10:00 a.m.	3	0	1	5	11:35 a.m.	1	0	0	2	4:15 p.m.	0	0	0	0
10:05 a.m.	1	0	1	5	11:45 a.m.	1	0	0	3	4:30 p.m.	0	0	0	2
10:10 a.m.	1	0	1	5	11:55 a.m.	0	0	0	3	4:50 p.m.	0	0	0	2
10:15 a.m.	1	0	0	5	12:05 p.m.	0	0	0	2	5:05 p.m.	0	0	0	2
10:20 a.m.	1	0	1	5	12:25 p.m.	0	0	0	4	5:25 p.m.	0	0	0	0
10:25 a.m.	1	0	0	5	1:00 p.m.	0	0	0	0					
10:30 a.m.	1	0	0	5	1:15 p.m.	0	0	0	3					

TABLE 10

Showing that KCN reduces the rate of oxygen consumption by Planaria agilis far more than does removal of the head. Worms 12 to 14 mm. in length; fed almost daily till the day before the experiment; 10 individuals in each bottle. Removal of heads performed within the half hour preceding the second test period. Each test period of 10 hours duration. Concentration of KCN, 0.000076 molecular. Temperature 19.6° to 19.7°C. Correction for 6 cc. of water displaced by rods and reagents: multiply by 1.048. 1 cc. thiosulphate = 0.1751 cc. oxygen at N. T. P.

PART	SET	BOTTLE	FIRST TEST (CONTROL)					SECOND TEST							
			Blanks					Blanks							
			cc. thio.	*average*				cc. thio.	*average*						
A	Blank (no worms present)	1	4.01	*4.04*				3.99	*4.00*						
		2	4.03					3.97							
		3	4.03					3.99							
		4	4.05					4.01							
		5	4.06					4.01							
		6	4.04					4.01							
			(1)		(2)	(3)		(4)		(5)	(6)		(7)	(8)	(9)
			Oxygen consumed		Weight of worms in each bottle	Oxygen consumption per gram per 24 hours		Oxygen consumed		Weight of worms in each bottle	Oxygen consumption per gram per 24 hours		Average rate of oxygen consumption in per cent of normal	Normal or headless	KCN or no KCN
			cc. thio.	*average*	*grams*	*cc. oxygen*	*average*	*cc. thio.*	*average*	*grams*	*cc. oxygen*	*average*	*per cent*		
B	I	1	0.53	*0.54*	0.0397	5.9	*5.8*	0.45	*0.48*	0.0396	5.0	*5.2*	(Normal)	Normal	No KCN
		2	0.54		0.0426	5.6		0.46		0.0421	4.8				
		3	0.54		0.0400	5.9		0.53		0.0394	5.9				

B	II	1	0.52	*0.52*	0.0430	5.3	*5.4*	0.21	*0.20*	0.0409	2.3	*2.2*	42	Normal	KCN
		2	0.55		0.0435	5.6		0.20		0.0420	2.1				
		3	0.49		0.0403	5.4		0.20		0.0389	2.3				
	III	1	0.57	*0.53*	0.0405	6.2	*5.9*	0.36	*0.35*	0.0377	4.2	*4.3*	83	Headless	No KCN
		2	0.52		0.0416	5.5		0.36		0.0360	4.4				
		3	0.50		0.0375	5.9		0.33		0.0331	4.4				
	IV	1	0.53	*0.51*	0.0411	5.7	*5.6*	0.15	*0.17*	0.0347	1.9	*2.1*	40	Headless	KCN
		2	0.50		0.0410	5.4		0.16		0.0353	2.0				
		3	0.49		0.0388	5.6		0.19		0.0329	2.5				
	V	1						0.20					Heads of Set III		No KCN
		2						0.21					Heads of Set IV		No KCN

fore, that the locomotion is stopped more completely by cutting off the head than by immersion in cyanide.

It remains, then, to determine the relative effects of cutting off the head and of immersion in cyanide solutions upon the oxygen consumption by Planaria. The experiment summarized in table 10 shows that 0.000076 molecular KCN reduced the oxygen consumption three times as much as cutting off the head. In other words, the cyanide had three times as much effect in inhibiting the oxidations in Planaria as could be attributed to loss of movement. Furthermore, there was still some movement in the cyanide solution..

This experiment involves an element not included in other experiments reported in this paper. When the head of a worm is cut off a considerable amount of tissue is removed, so that the amount of oxygen consumed after such removal cannot be compared with that before the removal, unless account is taken of the loss of body weight. In this experiment, therefore, the set of worms used in each bottle was weighed after each experimental period. The methods of weighing will be described in a later paper. Table 10, part A, gives the analyses of the blanks as heretofore. Part B, column 1, gives the oxygen consumption by the worms in each bottle during the control test. Column 2 gives the weight of the worms in each bottle and column 3 gives the oxygen consumption by these worms in cubic centimeters of oxygen gas at normal temperature and pressure per gram weight of worms per 24 hours. For the second test, columns 4, 5 and 6 give the corresponding data. The worms of set I were control worms, uncut and not in cyanide. They consumed 5.2 cc. of oxygen per gram per 24 hours during the second test. Set II were uncut worms in 0.000076 molecular KCN and they consumed 2.2 cc. of oxygen per gram per 24 hours, i.e., 42 per cent of the control or normal. Set III were headless worms, not in cyanide, and they consumed 4.3 cc. of oxygen per gram per 24 hours, i.e., 83 per cent of normal, twice as much as the normal worms in cyanide. Set IV were headless worms in cyanide. These had the same rate of oxygen consumption as the normal worms in cyanide. It may be concluded, therefore, that after eliminating its effect upon movement, KCN reduces the rate of oxidations at least 50 per cent, and when it is considered that the concentration of cyanide used in this experiment did not cause as great a decrease of the oxidations as the stronger solutions used in previous experiments, it may be concluded that, apart from its effect upon locomotion, KCN can inhibit the oxidations more than 50 per cent.

One further factor remains undetermined in these experiments. That is the relation of the loss of muscle tone in cyanide solutions to the reduction of the rate of oxidations. Headless worms contract strongly and it may be that they as well as the normal worms consume oxygen in maintaining a muscle tone which is lost in cyanide solutions.

SUMMARY

1. It has been proved that Winkler's method as carried out in these experiments is sufficiently reliable for the quantitative measurement of the oxygen consumption by Planaria.
2. The oxygen consumption by Planaria can be reduced to 30 per cent of the normal rate by KCN in a concentration as low as 0.0002 molecular.
3. The amount of the reduction varies with the concentration of the cyanide, not directly but as a power function, lower concentrations causing proportionately greater reduction than higher concentrations.
4. The relation between the concentration of KCN and its inhibitory effect upon the rate of oxidation in Planaria agilis indicates that a residual oxidation amounting to about 20 per cent of the normal cannot be inhibited by KCN.
5. No evidence was obtained of a stimulation of oxidations in low concentrations of KCN.
6. The rate at which Planaria absorbed oxygen in a cyanide solution was found to be practically the same during periods of different length from two to thirty-six hours. The effect of cyanide is therefore almost immediate and does not change materially with time.
7. The action of KCN upon oxidations in Planaria agilis is reversible, worms recovering their normal level of oxidations rapidly and completely after removal from cyanide solutions.
8. The inhibition of oxidations in Planaria by KCN could be attributed in only small part to the cessation of movement which results from the anesthetic action of cyanide.
9. These experimental results are considered important in themselves. Questions of their theoretical interpretation in relation to the toxic action of KCN and to the use of cyanide as a measure of the rate of oxidations are left for later consideration.

BIBLIOGRAPHY

(1) Child: Senescence and rejuvenescence, Chicago, 1915.
(2) Geppert: Zeitschr. f. klin. Med., 1889, xv, 208, 307.
(3) Schroeder: Jahrbücher f. wiss. Bot., 1907, xl, 409.
(4) Warburg: Zeitschr. f. physiol. Chem., 1910, lxvi, 305.
(5) Loeb and Wasteneys: Biochem. Zeitschr., 1911, xxxvi, 345.
(6) Loeb and Wasteneys: Biochem. Zeitschr., 1913, lvi, 295.
(7) Hyman: This Journal, 1916, xl, 238.
(8) Lund: This Journal, 1918, xlv, 365.
(9) Schönbein: Zeitschr. f. Biol., 1867, iii, 140.
(10) Kastle and Loevenhart: Amer. Chem. Journ., 1901, xxvi, 539.
(11) Loevenhart: Amer. Chem. Journ., 1903 xxix, 397, 663, 411.
(12) Loevenhart: Ber. deutsch. chem. Gesellsch., 1906, xxxix, 130.
(13) Matthews and Walker: Journ. Biol. Chem., 1909, vi, 29.
(14) Matthews and Walker: Journ. Biol. Chem., 1909, vi, 289.
(15) Lund: This Journal, 1918, xlv, 351.
(16) Sutten: Volumetric analysis, 1904, 287.
(17) Birge and Juday: Wisconsin Geol. and Nat. Hist. Survey, 1911, 16.

THE TREATMENT OF SEVERE AND PROGRESSIVE HEMORRHAGE BY INTRAVENOUS INJECTIONS

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It is generally conceded that transfusion with properly matched human blood is the best treatment for patients suffering from the effects of hemorrhage, when employed in conjunction with the usual measures of heat, rest, etc. The difficulty of making careful transfusions in the advanced dressing stations and ambulances has made necessary resort to infusions with solutions of sodium chloride, bicarbonate, gelatin, gum arabic and so on. The cases reaching large hospitals in the rear, where there would be more time and better facilities for transfusions, are rarely in a condition of shock. During a six months' period in a hospital for French wounded in Paris, the author saw no patient admitted in a condition of shock.

The experiments reported here were carried out at the suggestion of Dr. W. H. Howell, as part of the work being done under the supervision of the Committee on Shock of the Medical Division of the National Research Council.

In all cases dogs were used under ether anesthesia preceded by a grain of morphia. As a rule no ether was given during the period of low blood pressure. The anesthetic was administered from an ether bottle by means of a rubber tube passed through the larynx into the trachea. Aseptic precautions were observed throughout. One of the carotid arteries was connected with a mercury manometer so that the arterial blood pressure might be observed throughout the experiment and recorded on a smoked drum from time to time. A mean between systolic and diastolic pressure was used in the tabulation of results. The right femoral artery was connected by a cannula with a long rubber tube through which the animal could be bled at desired intervals. Washout cannulas were employed both in the carotid and femoral arteries so that the end of the artery, the cannulas and tubes could be washed clear with 2 per cent sodium citrate solution.

In the case of the femoral artery, the blood was washed out after each bleeding so that no difficulty was encountered from clotting. A cannula in the femoral vein was connected with tubing for infusions and for the determination of venous pressure.

For the arterial blood pressure, an arbitrary level was chosen (40 mm. Hg. in most cases). By successive bleedings the pressure was brought down to the chosen level and kept there for varying lengths of time. It happened in some cases that the pressure tended to fall below 40 mm. Hg. down to 35 or 30 mm. before the set time had elapsed. In such cases the pressure was maintained by infusions of warm salt solution. At the end of the period the pressure was raised by infusion of one of the solutions to be tested. The course of the blood pressure was then observed for several hours and the wounds closed. If the animal survived, his condition was observed for a week.

The solutions used, which were infused at about 38°C., were *a*, solution of sodium chloride 0.9 per cent; *b*, gum-bicarbonate solution according to the formula formerly recommended by the British Shock Committee (1) i.e., 6 per cent gum acacia and 2 per cent sodium bicarbonate. The two solutions, gum 9 per cent and bicarbonate 6 per cent, were sterilized separately in the autoclave and combined in the proportion of 2 to 1 just before infusion. The gum was made up in hot tap water, allowed to stand 24 hours, strained through flannel and filtered under pressure through a Buchner funnel. In the sterilization it was necessary to guard carefully against escape of gas from the bicarbonate by using strong air-tight bottles. When gas had escaped there appeared a white granular precipitate on combining the two solutions preparatory to use. *c*, Gum glucose solution as recommended by Erlanger (2). In these experiments the solution used contained 6 per cent gum acacia and 6 per cent dextrose. The amount of infusion, as a rule, was equal to the amount of blood withdrawn from the animal. The total volume of blood was calculated to be 5 per cent of the animal's weight, although it will be noted that two animals survived the loss of 101 per cent of the total thus calculated.

Much of the previous work on intravenous infusions has been done by relieving animals of a certain per cent of their calculated blood volume and following this immediately by saline or other injection. It seemed that such animals are not strictly comparable to patients who have lost blood and then suffer from the effects of the anemia and acidosis attendant upon continued low blood pressure; that is, patients who have entered into a state of shock. Also, the ability of different animals of the same species to withstand the same per cent of blood loss appears to vary greatly. It was therefore determined to bleed the animals until the arterial pressure reached 40 mm. Hg. and to maintain this pressure as nearly as possible by repeated hemorrhages or, if necessary, small saline infusions. At the end of this period of continued low blood pressure the solution to be tested was infused into the femoral vein.

Three animals were given injections of the sodium chloride solution after their arterial pressure had been about 40 mm. Hg. for less than forty-six minutes. All three recovered although they had lost 79 per cent, 69 per cent and 85.5 per cent respectively of their total calculated blood volume. It was thought that by exposing the animal to a longer period of low pressure a time would be found at which infusion of salt solution alone would not suffice to save it and that this period might then be used as a standard in testing other solutions. With this thought in mind the period of low blood pressure was increased up to seventy-five minutes at 40 mm. pressure and up to three and one-half hours at 50 mm. Of three dogs kept at 50 mm. pressure for two and one-half hours and infused with salt solution, two survived (experiments 22, 23, 24). Of two, kept for three and one-half hours at 50 mm. pressure, and then treated with salt solution, one survived (experiments 25, 26). The minimum time at which all animals would die when infused with salt solution was not determined, but a number of animals were subjected to the same treatment, along the lines stated above, and infused with one of the three solutions.

Selecting all the experiments in which the blood pressure was kept at 40 mm. for more than sixty minutes, we derive table 1. The number of experiments is rather small for drawing conclusions, but treatment with saline appears as successful as that with the other two solutions.

If one compares the results of the infusions according to the percentage of blood lost in each case table 2 is obtained.

It is difficult to draw conclusions as to the efficacy of the solutions employed from the above table also but it does show very plainly that large quantities of blood may be lost, if only it be replaced by an intravenous injection and the pressure raised thereby. If the adequate injection be delayed too long there comes a time when the organism will no longer respond, the pressure falls and respiratory failure supervenes. This period varies greatly in different animals. In experiment 27, 86 per cent of the total calculated blood was lost. Thirty-one minutes after the first bleeding the pressure had fallen to 26 mm. Hg. and 63 cc. saline solution were sufficient to raise it to 50 mm. After eighty-two minutes 52 cc. saline were sufficient to raise the pressure, which had again fallen, from 30 mm. up to 45 mm. Hg. At one hundred and twenty minutes 70 cc. saline raised the pressure from 27 to 44 mm.; one hundred and sixty-two minutes after the initial bleeding 88 cc. were required to raise the pressure from 24 to 38 mm. At two hundred and ten minutes the pressure dropped rapidly to 12 mm., and injection of gum-bicarbonate solution caused only an increased heart rate but did not prevent the developing respiratory failure nor influence the progressive fall of blood pressure to zero.

TABLE 1

AT END OF LOW BLOOD PRESSURE TREATED WITH:	NUMBER OF EXPERIMENT	PRESSURE MAINTAINED	DURATION OF LOW PRESSURE	RESULT	BLOOD LOST IN PER CENT OF THE TOTAL CALCULATED VOLUME
			minutes		*per cent*
*Salt solution	5	40 mm. Hg.	60	Died	90
Salt solution	6	40 mm. Hg.	64	Lived	93
Salt solution	9	40 mm. Hg.	75	Lived	94.5
				66⅔ per cent survival	
Gum-bicarbonate solution	10	40 mm. Hg.	60	Lived	101
Gum-bicarbonate solution	14	40 mm. Hg.	62	Lived	101
Gum-bicarbonate solution	12	40 mm. Hg.	75	Died	90
Gum-bicarbonate solution	11	40 mm. Hg.	89	Died	101
				50 per cent survival	
Gum-glucose solution	17	40 mm. Hg	62	Died	90
Gum-glucose solution	18	40 mm. Hg	62	Died	83
Gum-glucose solution	16	40 mm. Hg	63	Lived	87
Gum-glucose solution	15	40 mm. Hg.	70	Died	83
				25 per cent survival	

**Note.* No animal, treated with any infusion, lived after having lost more than 101 per cent of his total calculated blood volume. Hence, for purposes of comparison, experiments 2 and 7, where the blood loss was above that figure, are not included here.

While the animal has this low blood pressure, there results, as has been emphasized by Cannon (3), a condition of acidosis. This acidosis develops gradually as shown in experiment 13 (see fig. 1) in which the pressure was held at about 40 mm. Hg. for sixty-eight minutes. Determinations were made upon blood specimens with the Van Slyke apparatus:

Alkaline reserve before 1st bleeding	45.068
Alkaline reserve 3 minutes after 1st bleeding	38.429
Alkaline reserve 32 minutes after 1st bleeding	18.812
Alkaline reserve 61 minutes after 1st bleeding	13.078

TABLE 2

BLOOD LOST IN PER CENT OF TOTAL CALCULATED VOLUME	SOLUTION FOR INTRAVENOUS INFUSION	RESULT
per cent		
69.0	Saline	Lived
71.0	Saline	Lived
74.0	Saline	Lived
75.0	Saline	Died
78.0	Saline	Lived
79.0	Saline	Lived
81.0	Saline	Lived
82.0	Saline	Died
83.0	Gum-glucose	Died
83.0	Gum-glucose	Died
85.5	Saline	Lived
87.0	Gum-glucose	Lived
90.0	Saline	Died
90.0	Gum-glucose	Died
90.0	Gum-bicarbonate	Died
91.0	Saline	Lived
93.0	Saline	Lived
94.5	Saline	Lived
101	Gum-bicarbonate	Lived
101	Gum-bicarbonate	Died
101	Gum-bicarbonate	Lived
102	Saline	Died
109	Saline	Died
110	Saline	Died

At the time of the last determination the pressure was 26 mm. Hg. Eight minutes later the animal was dead in spite of intravenous injections with saline and gum-bicarbonate solutions.

In experiment 14 the dog survived a reduction of blood pressure to 40 mm. for sixty-two minutes; at the end of which time gum-bicarbonate solution was injected. The alkaline reserve varied as follows:

Alkaline reserve before 1st bleeding	38.221
Alkaline reserve 24 minutes after 1st bleeding	25.274
Alkaline reserve 61 minutes after 1st bleeding	20.579

It will be noted that in the fatal case the acidosis was much more rapidly developed.

For the sake of comparing the result of treatment by the three solutions, all experiments in which the alkaline reserve was determined are tabulated.

TABLE 3

TREATED WITH SALINE SOLUTION			GUM-BICARBONATE SOLUTION			GUM-GLUCOSE SOLUTION		
Number	Alkaline reserve before injection	Result	Number	Alkaline reserve before injection	Result	Number	Alkaline reserve before injection	Result
24	22.900	Lived	12	24.057	Died	16	20.239	Lived
22	22.322	Lived	14	20.579	Lived	17	19.222	Died
21	17.517	Lived	28	16.749	Lived	18	18.345	Died
25	13.500	Died	13	13.078	Died	15	15.105	Died
23	12.812	Died						

With the exception of no. 12, in which there was a fatal outcome with a comparatively high alkaline reserve, it will be noted that all deaths occurred in animals whose reserve was low. If the degree of shock be measured by the amount of acidosis, gum-glucose solution appears the least useful while gum-sodium-bicarbonate solution appears to be no more efficacious as an infusion than saline.

In a number of the experiments a venous manometer was used to measure the height of water pressure necessary to cause a flow of saline solution in the femoral vein toward the auricles. In every case zero for these readings was the level of the femoral vein, this point being accurately determined by breaking the connection of tubing and venous cannula and allowing the liquid in the pressure column to fall to the level of the vein. With this point as zero, readings of venous pressure were taken at intervals of five to fifteen minutes. Whenever the pulse and respiration caused a corresponding rise and fall in the pressure column, while a determination was being made, the lowest point to which the pressure fell was recorded.

In all fatal cases observed the continued fall of arterial pressure was accompanied not by a fall of venous blood pressure but by a rise. In every case where a fall of arterial pressure was accompanied by a rise of venous pressure for twenty minutes or more the outcome was fatal regardless of treatment. Figures 1, 2 and 3 will serve to illustrate this point. In eleven experiments the venous pressure was recorded at frequent intervals. Of the animals thus used seven died. In

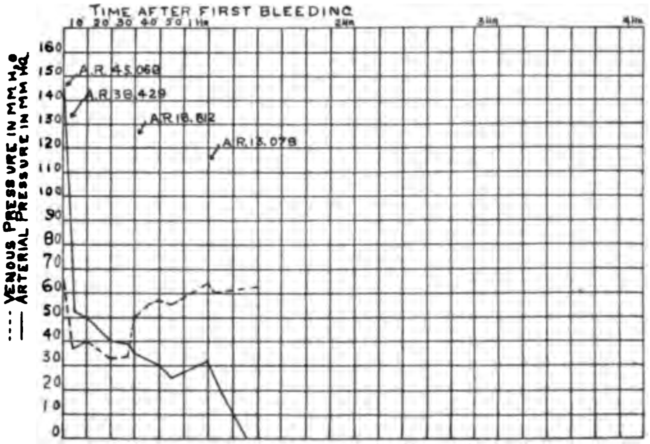

Fig. 1. Experiment 13; 100 per cent of total calculated blood volume removed. After 25 minutes the venous pressure rose steadily, the last reading being taken just before death. The corresponding increase of acidosis is measured by the diminishing alkaline reserve (A. R.) S–1 Infusion of 200 cc. normal saline.

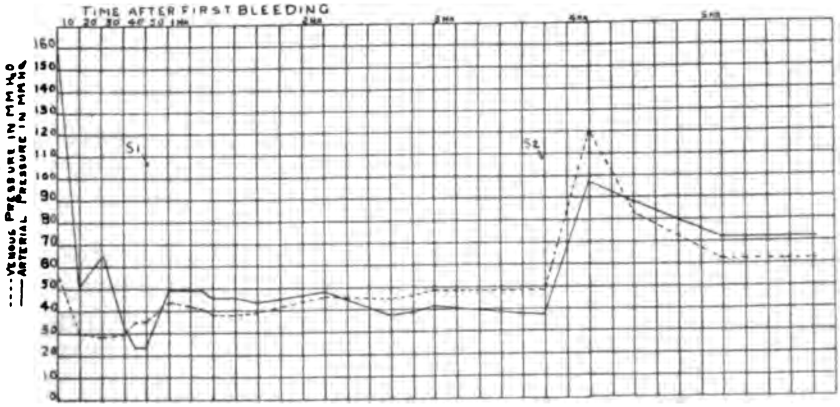

Fig. 2. Experiment 26; 71 per cent of the total calculated blood volume removed. In this case the dog recovered completely and there was no rise of venous pressure independent of arterial pressure. Infusions of: S–1, 68 cc. saline solution; 438 cc. saline solution.

every fatal case except one there was a period of twenty minutes or more before the infusion when the arterial pressure was falling and the venous pressure rising. The exception, experiment 18, had arterial and venous pressures which corresponded closely throughout. This animal, unlike the others, lived through the first twenty-four hours to succumb on the second day. Of the four which survived none had any considerable rise of venous pressure associated with falling arterial pressure. In these experiments, then, every fall of arterial pressure associated with a rising venous pressure for more than twenty minutes had a fatal outcome, and every experiment with a fatal outcome inside of twenty-four hours had such a fall of arterial pressure associated with rising venous pressure.

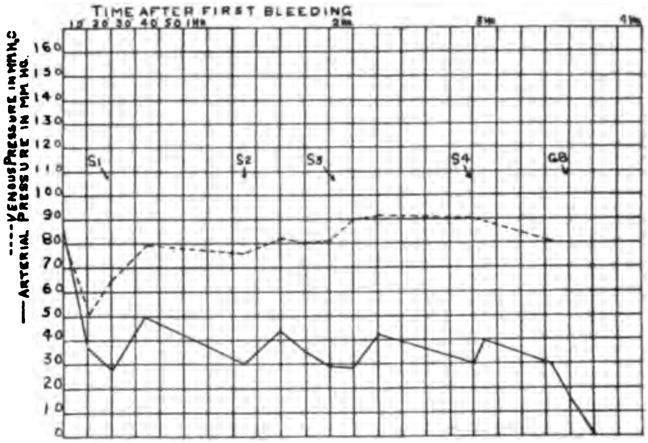

Fig. 3. Experiment 27; 86 per cent of total calculated blood removed. Infusions of: S–1, 62 cc. saline; S–2, 52 cc. saline; S–3, 70 cc. saline; S–4, 88 cc. saline; G-B, gum bicarbonate solution.

The question naturally arises: by what means are these arterial and venous changes associated? Loss of tone somewhere in the periphery, e.g., arteries, capillaries or venules, might seem to satisfactorily explain the approach of the two pressures toward each other. The following experiment is suggestive of the cause of the changes in pressures (see fig. 5). A dog was prepared so that the arterial and venous pressures could be recorded in the right femoral artery and vein. One carotid was cannulated so that blood could be drawn off. Cannulas were placed in a branch of the left femoral artery and of the left femoral vein so that, on clamping the main artery and vein proximally, the vascular bed of the leg might be perfused with normal salt solution under a constant pressure and the amount of fluid passing through the leg in a given period measured. Then by clamping the cannulated branches and removing the clamps from the main vessels the leg could be again restored to the general circulation. The animal was bled, as in the previous experiments, so as to maintain the blood pressure at about 40 mm. Hg. Before the bleeding, 23.5 cc. of saline were perfused through the leg in one minute under a pressure equal to that in the femoral artery of the other leg. After six minutes of low blood pressure, only 21.5 cc. of saline could be perfused in one minute under the same pressure, showing that there had been an increase in peripheral vascular tone. Following the first bleeding the venous pressure had fallen with the arterial pressure (fig. 5). After seventy-six minutes of low blood pressure, 28 cc. could be perfused. There had evidently been a loss of tone in the peripheral vessels since the last perfusion and reference to figure 5 makes it evident that the venous pressure had risen while the arterial pressure had fallen in that in-

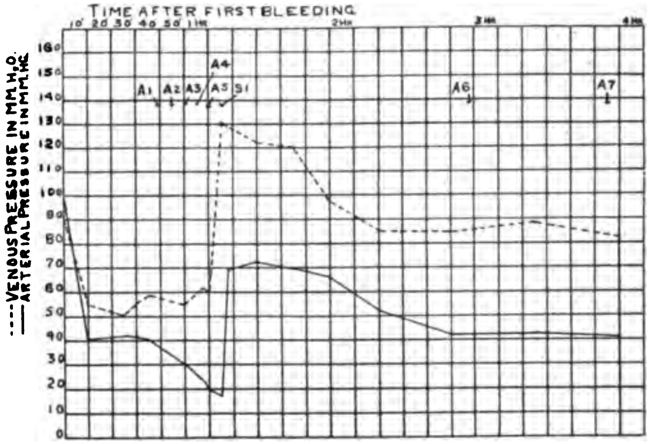

Fig. 4. Ninety-four per cent of total calculated blood volume lost. Low blood pressure maintained for one hour. Death occurred four hours after the infusion of saline solution. Atropine was injected intravenously with 1–4 cc. of normal salt solution.

A–1, 0.001 gm. atropine; A–2, 0.001 gm. atropine; A–3, 0.001 gm. atropine; A–4, 0.002 gm. atropine; A–5, 0.005 gm. atropine; A–6, 0.002 gm. atropine; A–7, 0.002 gm. atropine. S–1, 432 cc. normal salt solution.

terval. One hundred and thirty-two minutes after the first bleeding 36.5 cc. were perfused. The loss of vascular tone which is here indicated by the increased rate of flow through a peripheral vascular bed under constant pressure conditions might account for both falling arterial pressure and rising venous pressure. The data at hand throw no light on the condition of the heart, a progressive enfeeblement of which would contribute to the pressure changes observed. In this same experiment (no. 30), the pressure fell progressively to 4 mm. Hg., when respiratory failure supervened. At this point warm, normal saline solution was injected into a vein and the heart, which had almost stopped, began to beat more forcibly. Its rhythm and amplitude of contraction, as well as may be estimated by the mercurial manometer, came back to what they had been at the beginning of the experiment and the pressure rose to 8 mm. Hg. for a few minutes. This would seem to indicate that not cardiac failure, but failure of venous return is the primary factor in the fatal result. The blood is evidently lost or pooled in the relaxed peripheral vessels.

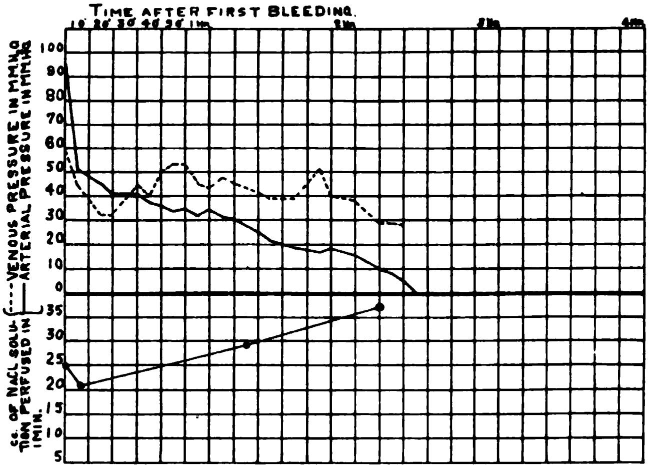

Fig. 5. Perfusion experiment. Blood loss was 61 per cent of the total calculated blood volume plus the unknown amount of blood washed out each time the hind limb was perfused. The vascular bed of this hind limb formed part of the general circulation except for four brief intervals when it was perfused with normal saline solution.

Reid Hunt (4) in working on the action of acetyl choline as a vasodilator and depressor of blood pressure has shown that everywhere its action is opposed by that of atropine. In view of the presence of choline as one of the products of metabolism it seemed advisable to inquire into the effect of atropine upon an animal when in a state of low blood pressure following hemorrhage (see fig. 4). The immediate effect of atropine upon the arterial pressure after each injection was in every case a very slight rise followed by a somewhat greater fall. The dog was infused with normal saline solution after the arterial pressure had been about 40 mm. Hg. for sixty-five minutes. The pressure rose to 73 mm. after the infusion but it was not necessary to readminister ether as it had been in most experiments. The pupils were dilated, the corneal reflexes present. Faradic stimulation of the central end of one vagus,—the other being intact—caused a period of apnoea but had no effect upon the heart rate. The animal died four hours after the saline injection, apparently deriving no benefit from the atropine.

In five experiments the saphenous nerve was stimulated at the end of the low blood pressure period, the stimulation being either electrical (induction shocks) or mechanical. In two, the respiration increased and the animal moved. In one, only the respiration was affected. Thirty minutes later this animal was dead. Bulbar reflexes seemed to be still active. In the two cases where a vasomotor reaction resulted some central control of the vasomotor system remained, although in one (exp. 10) the state of shock was so profound that the animal never roused from it.

Eight experiments similar to those described in this paper were carried out upon dogs that had been used for demonstration purposes, after two to six hours of ether anesthesia. The vagi had been cut and the saphenous nerve stimulated during the demonstration. In these animals a loss of 40 to 60 per cent of the calculated volume of blood brought about changes comparable to those we have noted above in animals losing 75 to 90 per cent.

SUMMARY AND CONCLUSIONS

The blood pressure of experimental animals was kept at a shock level for various lengths of time by progressive bleeding so that their condition resembled that which must frequently exist in wounded men at the front who have sustained severe and continued loss of blood. After analyzing the condition of these experimental animals according to the duration of low blood pressure, the amount of blood lost and the degree of acidosis resulting, I have not been able to show that gum-sodium bicarbonate solution or gum-glucose solution is more efficacious in saving life than is an isotonic solution of sodium chloride.

By perfusing an isolated vascular bed it has been shown that following a large hemorrhage there is a primary constriction of the peripheral vessels. With continued low blood pressure this constriction passes off and dilatation supervenes. Associated with this dilatation the venous pressure rises while the arterial pressure is falling. All dogs in which the venous pressure rose while the arterial pressure was falling, for a period of twenty minutes or more, died regardless of treatment.

The effects of stimulation of the saphenous nerve while these animals were in a condition of profound shock indicated that the reflexes affecting respiration and blood pressure were not paralyzed, although this point deserves more careful investigation.

BIBLIOGRAPHY

(1) Memorandum I of Reports of Special Investigation Committee on Surgical Shock and Allied Conditions, November 25, 1917.
(2) Erlanger and Gasser: Report to Sub-Committee on Shock, Committee on Physiology, Med. Div., Natl. Research Council.
(3) Cannon: Journ. Amer. Med. Assoc., 1918, lxx, 531.
(4) Hunt: This Journal, 1918, xlv, 197.

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THE REASON FOR THE SPECIFIC DYNAMIC ACTION OF PROTEIN

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Lavoisier (1) showed that the ingestion of food increased oxidation in the body. Rubner (2) found that of the food materials, the ingestion of meat increased oxidation most, fat next and sugar least. Rubner designated the action of protein in increasing oxidation more than the other food materials as the *specific dynamic action of protein.* Several theories have been advanced in attempts to explain how the ingestion of food increases oxidation. The theory, or some modification of the theory of Voit, who claimed that the presence of food augmented the inherent power of the cells to metabolize, is the one that has met with most general acceptance. Lusk (3) originally attributed the increase in metabolism following the ingestion of meat, to the mass action of the amino acids acting as stimuli on the protoplasm of the cells. Later, (4) however he modified this explanation and attributed the increase in oxidation to the stimulating action of the organic acids resulting from the deamination of the amino acids, while Grafe (5) attributed the stimulating action of protein to the amino groups. We (6) found that the increase in oxidation produced by the ingestion of food, as well as by a variety of ways, was accompanied by an increase in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide. We also found that meat, in keeping with its greater stimulating action on heat production, produced a greater increase in catalase than the other food materials, hence the conclusion was drawn that the increase in oxidation produced by the ingestion of food as in other ways, was due to an increase in catalase which was brought about by the stimulation of the alimentary glands, particularly the liver, to an increased output of this enzyme.

Lusk found that the ingestion of amino acids, such as glycocoll and alanin, produced an increase in oxidation while glutamic acid did not. Grafe, on the contrary, found that the ingestion of glutamic acid as well as of glycocoll and alanin produced an increase in oxidation. We (6) found that the ingestion of glycocoll and alanin produced an increase in catalase and that glutamic acid did not. This fact was offered as an explanation for Lusk's observation that the ingestion of glycocoll and alanin increased oxidation while glutamic acid did not. Grafe (5) found that ingestion of asparagin, aspartic and succinic acids and acetamid produced an increase in oxidation. Recently Lusk (7) has repeated and confirmed his original work, extending it to include the effect of asparagin, aspartic and succinic acids, acetamid and ammonium citrate on heat production and found, contrary to Grafe, that these substances produced no increase in oxidation.

One of the objects of the present investigation was to determine if the ingestion of aspartic and succinic acids, acetamid and asparagin would or would not produce an increase in catalase. The animals used were dogs. After etherizing the animals and opening the abdominal wall each of these substances was introduced in about equal quantities into the stomach and upper part of the intestines. The amounts of the substances will be given under the individual experiments. The catalase in 0.5 cc. of blood taken from the portal and jugular veins and from the liver was determined before as well as at fixed intervals after the introduction of the materials. The determinations were made by adding 0.5 cc. of blood to 50 cc. of hydrogen peroxide in a bottle at approximately 22°C. and the amount of gas liberated in ten minutes was taken as a measure of the catalase content of the 0.5 cc. of blood.

In figure 1 are shown the effects of the introduction of glutamic, aspartic and succinic acids and of asparagin on catalase production. The acids were given in the form of sodium salts and in saturated solutions. The figures along the ordinate (0 to 100) indicate amounts of catalase measured in cubic centimeters of oxygen and those along the abscissae, time in minutes. The continuous line curves show amounts of catalase in the blood of the liver; the discontinuous line curves, amounts in the blood of the portal vein; and the dotted line curves, the amount of catalase in the blood of the jugular vein. It may be seen that the introduction of 14 grams per kilo of body weight of glutamic acid produced an increase in catalase as is indicated by the increase in the amount of

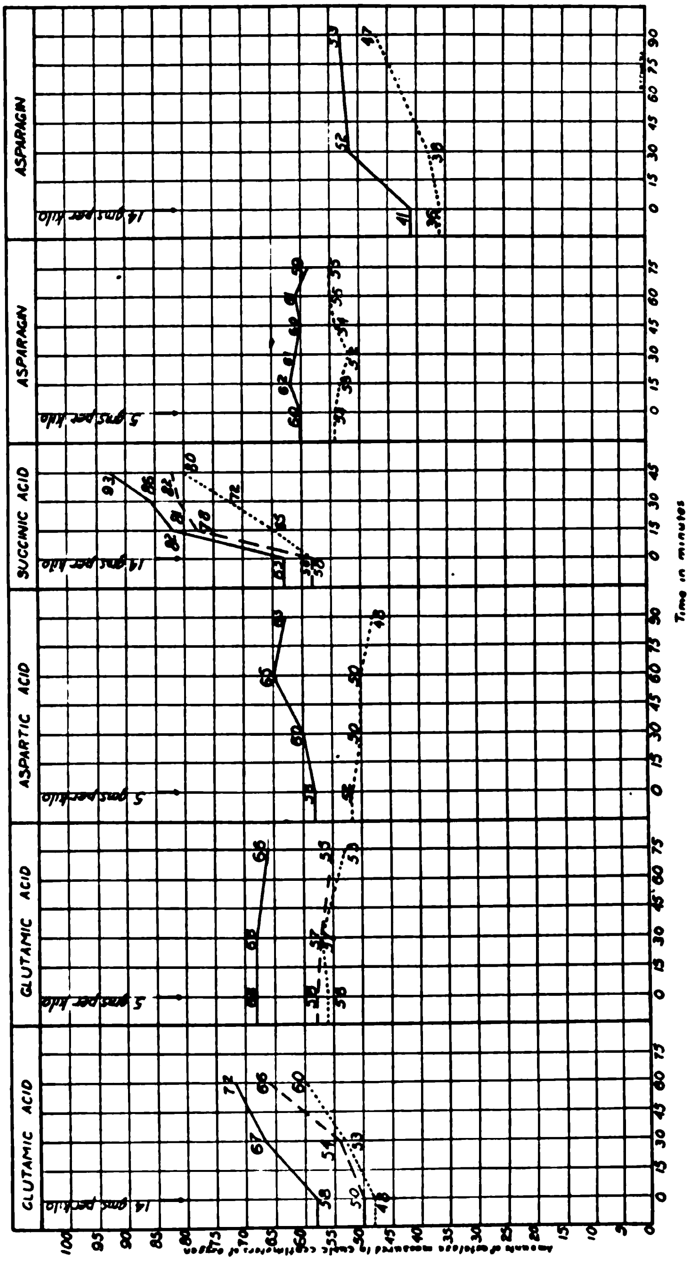
GLUTAMIC ACID
14 gms per kilo
GLUTAMIC ACID
5 gms per kilo
ASPARTIC ACID
5 gms per kilo
SUCCINIC ACID
14 gms per kilo
ASPARAGIN
5 gms per kilo
ASPARAGIN
14 gms per kilo
0 15 30 45 60 75 90
100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0
Time in minutes

Fig. 1

oxygen liberated from hydrogen peroxide by 0.5 cc. of blood, while the introduction of 5 grams per kilo produced no increase. The fact that the catalase of the blood of the liver was increased particularly during the first fifteen minutes much more than that in the blood of the portal or jugular veins is taken to mean that the glutamic acid was stimulating the liver to an increased output of this enzyme. Under aspartic acid it may be seen that 5 grams per kilo of this acid produced no increase in catalase. The effect of larger amounts of this acid could not be studied owing to the fact that we had no more and could procure none. However, the effect of a large amount of a closely related substance, succinic acid, was tried and it may be seen that 14 grams per kilo of this acid produced a great increase in catalase. It should be mentioned in this connection that a small amount of succinic acid, about 1 gram per kilo, which Lusk found to produce no increase in oxidation, was used and found to produce no increase in catalase. Under asparagin it may be seen that 5 grams per kilo did not produce an increase in catalase while 14 grams per kilo did. Since small amounts of glutamic, aspartic and succinic acids and asparagin produced no increase in catalase in keeping with Lusk's observation that small amounts of these substances produce no increase in oxidation, whereas large amounts produced an increase in catalase, in keeping with Grafe's findings that large amounts of these substances produce an increase in oxidation, it is probable that the difference in the amounts of the substances used may account for the difference in Lusk's and Grafe's results.

In figure 2 are shown the effects of the introduction into the stomach and intestines of glycocoll, acetamid, sodium acetate, olein, potassium oleate and dextrose. The manner of introduction of the substances into the alimentary tract and of the determination of the catalase was the same as that already described. The amount of glycocoll, acetamid, sodium acetate and dextrose used was 20 grams per kilo and the amount of olein, glycerin and potassium oleate was 10 grams per kilo. All these materials were given in a saturated solution. All the substances produced an increase in catalase, as is indicated by the increase in the amount of oxygen liberated from hydrogen peroxide by 0.5 cc. of blood. In a previous publication we found that the introduction of sodium palmitate produced no increase in catalase, whereas in the present investigtion we find potassium oleate does. In looking into the difference in solubility of these two soaps, it was found that potassium oleate is about twelve times more soluble than sodium palmitate and hence was absorbed, presumably more rapidly and more extensively, than sodium

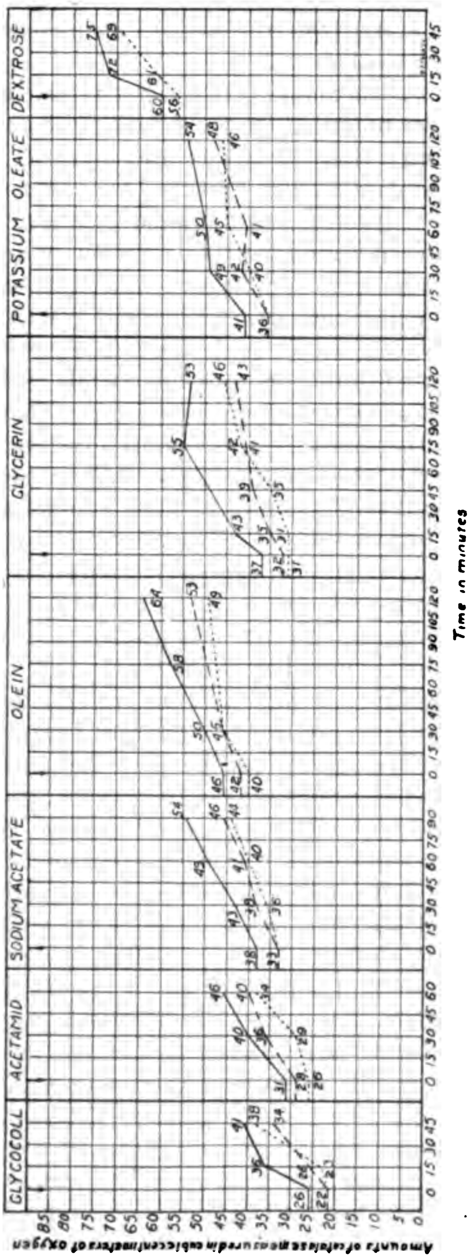
GLYCOCOLL
ACETAMID
SODIUM ACETATE
OLEIN
GLYCERIN
POTASSIUM OLEATE
DEXTROSE
Amounts of catalase measured in cubic centimeters of oxygen
85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0
Time in minutes

Fig. 2

palmitate and for this reason it is assumed that the potassium oleate was more effective in stimulating the liver to an increased output of catalase than was sodium palmitate.

In figure 3 is given the maximum percentage increase in the catalase of the blood of the liver produced by the different substances given in figure 2. It may be seen that the amino acid, glycocoll, produced 57 per cent increase in catalase, sodium acetate 42 per cent and acetamid 48 per cent increase. Glycocoll and acetamid produced a greater increase in catalase than did sodium acetate. By comparing the formulae of these three substances it may be seen that all three are derived from acetic acid; the amino acid glycocoll (CH_2NH_2COOH) and acetamid (CH_3CONH_2) being acetic acid (CH_3COOH) with an amino (NH_2) group introduced into the molecule, while sodium acetate (CH_3COONa) has the element sodium introduced. Hence the effect of the introduction of the amino (NH_2) group into the acetic acid molecule was to increase the effectiveness of this molecule in stimulating the liver to an increased production of catalase and hence an increase in oxidation. If the introduction of the amino (NH_2) group into the organic acids, propionic, valerianic, caproic, succinic and glutaric, also increases the effectiveness of these acids in stimulating the liver to an increased output of catalase, this fact may explain the great increase in heat production after the ingestion of protein.

The curves given in figure 3 show that the introduction of olein, a fat, into the alimentary tract produced 40 per cent increase in the catalase of the blood of the liver, glycerin 48 per cent and potassium oleate 32 per cent. Glycerin produced a greater increase in catalase than did olein and potassium oleate produced a smaller increase. By comparing the formulae of these substances it will be seen that the fat, olein $(C_{17}H_{33}COO)_3C_3H_5$ has in its molecule a part of the glycerin $C_3H_5(OH)_3$ molecule and a part of the oleic acid ($C_{17}H_{33}COOH$) molecule. Since oleic acid or potassium oleate produces a smaller increase in catalase than the olein, and glycerin a larger increase, it follows that the effect of the glycerin radical in the olein molecule was to augment the effectiveness of this molecule in producing an increase in catalase in a manner similar to but not so extensive as the amino (NH_2) group in the amino acid, glycocoll. The sugar, dextrose, ($C_6H_{12}O_6$), produced a smaller increase in catalase than the fat or proteim, in keeping with the fact that the ingestion of sugar produces smaller increase in oxidation than fat or protein. It is assumed that glucose amine ($C_6H_{11}NH_2O_5$) would be more effective in stimulating the liver to an increased production of catalase and hence in producing an increase in oxidation than glucose ($C_6H_{12}O_6$) owing to the presence of the amino (NH_2) group in the molecule. We were unable to procure any glucosamine and hence could not try out its effect on catalase production.

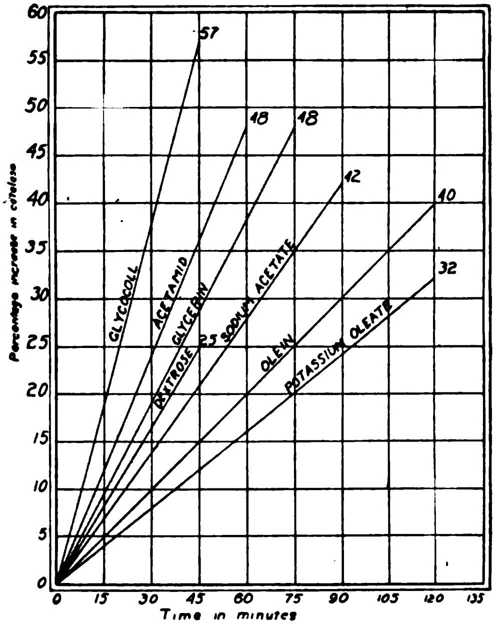

Fig. 3

SUMMARY

The introduction of small amounts of glutamic, aspartic and succinic acids, asparagin and acetamid produces no increase in catalase in keeping with Lusk's observation that small amounts of these substances do not increase oxidation. On the contrary, large amounts do produce an increase in catalase, in keeping with Grafe's observation that large amounts increase oxidation.

Evidence is presented to show that the amino (NH_2) group in the protein molecule renders protein, or meat, a more effective stimulant to catalase production and hence to heat production than fat and that the glycerine radical in the fat molecule renders fat more effective in this respect than sugar.

BIBLIOGRAPHY

(1) **Lavoisier**: Mem. de l'Acad. des. Sci., 1780.
(2) **Rubner**: Energiegesetze, 322.
(3) **Lusk**: Journ. Biol. Chem., 1912, xiii, 27, 155.
(4) **Lusk**: Journ. Biol. Chem., 1915, xx, 555.
(5) **Grafe**: Deutsch. Arch. f. Klin. Med., 1915, cxviii, 1.
(6) **Burge and Neill**: This Journal, 1918, xlvii, 13; 1918, xlvii, 351.
(7) **Lusk**: Journ. Biol. Chem., 1918, xxxvi, 2, 415.

ON THE STABILITY OF ESTERASE IN GROUND LIVER PRESERVED IN GLYCEROL

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In a series of experiments previously reported (1) the esterase content of the liver, spleen and kidneys in certain experimentally produced pathologic conditions was studied. These ground-up organs have now been standing in glycerol in a dark place, well corked, for from twenty-two to thirty-four months. The esterase content of the livers has again been tested, using the same dilutions of the extract as before. The results of these experiments are shown in table 1, from which it is evident that none of the specimens of ground-up liver had completely lost their esterolytic power.

The results may again be tabulated according to whether there has been *a*, an increase; *b*, no change; or *c*, a decrease in the ester-splitting capacity of the extracts. This is also shown in table 1. Of 22 extracts, 9 showed an increase, 7 showed a decrease and 6 showed little or no change in the amount of 0.1 N sodium hydroxide solution required to neutralize the acid liberated by the enzyme from ethyl acetate. It is evident, therefore, that at least for a period of thirty-four months the time element is not the most important factor in determining the diminution of esterase in ground liver preserved in glycerol.

The greatest increases noted were in extract IIIa from 3.45 cc. of 0.1 N sodium hydroxide in the first titration to 4.60 cc., and in control 3, from 3.45 cc. 0.1 N sodium hydroxide in the first titration to 4.80 cc. in the last titration after thirty-two and thirty-two and one-half months, respectively, in glycerol. The most marked decreases were shown by extracts Ia from 2.50 cc. 0.1 N sodium hydroxide to 1.15 cc. in the titration after thirty-two months in glycerol; and by sugar-fed 2 from 4.80 cc. 0.1 N sodium hydroxide to 2.60 cc. in the last titration after twenty-three and one-half months in glycerol.

TABLE 1

Showing the esterase content of ground-up liver preserved in glycerol for 21 days, and 22 to 34 months, respectively, expressed in cubic centimeters of 0.1 N sodium hydroxide required to neutralize the acid liberated from ethyl acetate

INCREASE			No change			DECREASE		
Animal	First titration	Last titration	Animal	First titration	Last titration	Animal	First titration	Last titration
In glycerol for from 30 to 34 months								
Control 1	3.35	3.80	Gl. 2	4.75	4.65	Chl. 2	3.40	2.90
Control 2	3.25	3.75	Pep. 2	4.90	4.90	Ib	3.55	3.20
Control 3	3.45	4.80	Ana. 1	3.90	4.00	Ia	2.50	1.15
Control 4	3.20	3.70	Ana. 2	4.10	4.00			
Gl. 1	4.60	5.00						
IIIa	3.45	4.60						
In glycerol for from 22 to 24 months								
IIb	4.15	4.40	IIc	4.30	4.35	S.F.1	4.60	4.10
Id	3.90	4.20	IId	3.75	3.65	IIa	4.40	3.95
Pep. 1	3.80	4.00				Ic	3.35	2.30
						S.F.2	4.80	2.60

The relation of the stability of the esterase in glycerol extracts of ground-up liver to the experimental procedure employed is shown in table 2.

DISCUSSION

It is evident from the above tables that ground-up liver preserved in glycerol may retain its esterolytic power unimpaired for a period of at least thirty-four months. The esterase content of the clear filtered glycerol extracts of liver may even be greater after many months' extraction than after the standard twenty-one days adopted in the previously reported experiments. That this was not due to any increased concentration resulting from evaporation was evident from the absence of any demonstrable loss of volume of the mass of tissue and glycerol.

The esterase in these livers is an intracellular enzyme. Duccheschi and Almagia (2) believe that cells yield their "lipase" (esterase) with some difficulty. It would appear reasonable, therefore, to suppose that the liberation of the enzyme might be related in a quantitative way to the autolysis of the tissues being extracted. No tests were made to determine chemically the amount of autolysis that had taken place in these ground-up livers. In no instance was there complete liquefaction of the mass although it did appear to be softer and more finely divided after the prolonged extraction than after twenty-one days. There is evidence in numerous experiments recorded in the literature which jus-

TABLE 2

Showing the relation of the stability of esterase in glycerol extracts of liver to the pathologic condition experimentally produced

ANIMAL	FIRST TITRATION		LAST TITRATION	
	Days in glycerol	0.1 NaOH	Months in glycerol	0.1 NaOH
		cc.		*cc.*
Control 1	20	3.35	34½	3.80
Control 2	21	3.25	34	3.75
Control 3	21	3.45	23½	4.80
Control 4	21	3.20	34	3.70
Phosphorus poisoning Ia	21	2.50	32	1.15
Phosphorus poisoning Ib	22	3.55	31	3.20
Phosphorus poisoning Ic	21	3.35	22½	2.30
Phosphorus poisoning Id	20	3.90	22½	4.20
Phosphorus and sugar IIa	20	4.40	23½	3.95
Phosphorus and sugar IIb	20	4.15	23½	4.40
Phosphorus and sugar IIc	20	4.30	23½	4.35
Phosphorus and sugar IId	20	3.75	22½	3.65
Sugar-fed 1	23	4.60	23½	4.10
Sugar-fed 2	20	4.80	23½	2.60
Sugar-fed Gl. 1	21	4.60	32	5.00
Sugar-fed Gl. 2	21	4.75	32	4.65
Phosphorus and sugar recovered IIIa	21	3.45	23½	4.60
Chloroform 2	21	3.40	30½	2.90
Peptone poisoning 1	25	3.80	24	4.00
Peptone poisoning 2	21	4.90	30	4.90
Peptone poisoning 3	22		34	3.95
Anaphylactic shock 1	22	3.90	31	4.00
Anaphylactic shock 2	22	4.10	31	4.00

tifies the belief that autolysis proceeded at different rates in the several groups of livers used in these experiments. Jacoby (3), Wells (4) and others have shown that phosphorus- and chloroform-poisoned livers autolyze much more rapidly than do normal livers. Evidence will be presented elsewhere (5) which appears to show that the rate of autolysis of liver is inversely proportional to the amount of glycogen present in the organ. A study of table 2 does reveal a relationship between the probable rate of autolysis in a given specimen and the increase or decrease of the esterolytic power of the extract. Thus, of four animals poisoned with phosphorus and whose livers might be expected to autolyze more completely, three showed a definite decrease in esterase content of the extracts; while of four dogs which were fed considerable quantities of sugar, whose livers contained large amounts of glycogen and would therefore autolyze more slowly, only one showed a decrease in esterolytic activity. Of four animals poisoned with phosphorus and fed with sugar, only one showed a decrease in esterase after prolonged extraction.

However, it does not necessarily follow that glycogen has a preservative action upon the esterase in prolonged extraction of ground-up liver in glycerol, for the conduct of the extracts of livers of control animals did not differ materially from that of the livers of sugar-fed dogs. But there is indication that the tendency to lose esterase is somewhat greater in the glycerol extracts of livers of sugar-fed dogs than in those of the controls.

In the previous paper (1) experiments were cited showing the inhibitory effect of bile upon the action of esterase. It seems probable, therefore, that the greater loss of esterolytic power in the livers of phosphorus-poisoned dogs may be related to the amount of bile present in the extracts which were definitely more deeply bile-stained than any of the others. This is indicated also by the fact that the greatest loss of esterase was in the liver of dog Ia which, in the earlier experiments, showed a lower esterase content than that of any other phosphorus-poisoned animal. This was explained in the previous paper as the probable result of the presence of the large amount of bile in the extract.

The extract of the liver of one of the sugar-fed dogs (sugar-fed 2) also showed a very marked loss of esterolytic power after twenty-three and one-half months in glycerol. No satisfactory explanation is offered for this apparent anomaly. If the results obtained from the extract of the liver of this animal are omitted, it is seen from table 2 that the average ester-splitting power of the livers of sugar-fed dogs is still greater than that of the livers of the control animals, although the difference is not so great as in the case of the twenty-one-day extracts used in the experiments previously reported.

CONCLUSIONS

1. Ground dog's liver preserved in glycerol may retain its ester-splitting power unimpaired for a period of at least thirty-four months.
2. In some instances the clear filtered extract of the liver of a given animal shows a greater esterase content after prolonged standing in glycerol than after extraction for a period of twenty-one days. This increase is independent of the probable rate of autolysis of the specimen.
3. Diminution in the esterolytic power of the clear extracts is not related to the time during which the tissues have stood in glycerol, but to some other factor, possibly to the amount of bile present.
4. The feeding of sugar and the storage of the glycogen of the liver increases the ester-splitting activity of that organ.

BIBLIOGRAPHY

(1) SIMONDS: Journ. Exper. Med., 1918, xxviii, 663.
(2) DUCCHESCHI AND ALMAGIA: Arch. ital. Biol., 1903, xxxix, 29.
(3) JACOBY: Zeitschr. physiol. Chem., 1900, xxx, 174.
(4) WELLS: Journ. Amer. Med. Assoc., 1906, xlvi, 341.
(5) SIMONDS: To appear in Arch. Int. Med., 1919.

THE MECHANISM OF ETHER HYPERGLYCEMIA

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In the course of studies on the relation of the hypophysis to hyperglycemia conducted by one of us (1), the influence of the ether anesthesia was the most difficult question to evaluate in the interpretation of the results. In the light of the existing literature, the influence of the anesthetic seemed of sufficient importance to warrant further investigation. Most of the data of the present paper was collected at that time but remained unpublished because of pressure of other work.

The ether glycosuria previously noted by Hawk (2) was shown to be associated with a hyperglycemia by Underhill (3) and Seelig (4). Seelig concluded that the glycosuria depends on quantity of ether used and duration of time through which it is administered, that it occurred regularly in animals fed on meat and in animals on carbohydrate diet only if anesthetic was administered within ten hours of last meal. Intravenous administration of oxygen prevented the appearance of glycosuria.

King (5) and associates reviewed the literature rather completely and made the first attempt to analyze the mechanism involved. They confirm the findings of Seelig that the depth and length of anesthesia are important modifying factors. In a later investigation King, Moyle and Haupt (6) induced anesthesia by the intravenous administration of ether in salt solution, and concluded that the site of action of ether is on the liver cells. A more detailed consideration of their findings will be presented later.

The more recent colorimetric methods of estimating blood sugar offer such advantages over the Oppler-Bertrand (7) method employed in these investigations that a repetition of a few of their experiments seemed to us advisable.

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[1] The greater part of the experimental work of this paper was collected while both authors were members of Department of Pharmacology, Northwestern University Medical School.

Stewart and Rogoff (8) were able to produce hyperglycemia in animals by means of ether, asphyxia and so-called emotional states without the liberation of adrenalin in quantities sufficient to detect.

The mechanism of the hyperglycemia associated with the stimulation of the splanchnic nerve has been quite completely investigated by Macleod (9) and his coworkers. It furnishes an excellent basis for attacking the problem in hand. Stimulation of the splanchnic nerve gives a hyperglycemia. Stimulation of the hepatic nerves in the hepatic pedicle also gives a hyperglycemia if the adrenals are intact. If they have been removed from the circulation by ligatures the rise does not occur. The stimulation does not affect the quantity of glycogenase present. There is no absolute evidence to show whether these fibers are to be regarded as secretory or accomplish their effect through vasomotor changes. A small amount of adrenalin in the circulation appears to be a necessary factor in their activity.

Methods. The established effect of even slight degrees of asphyxia on the level of glycemia made it necessary at the outset to adopt all possible precautions against its occurrence. Unfortunately in a normal animal when an initial high level is once established it is not a transitory phenomenon but continues throughout the experiment.

The methods available for reducing a dog to the state of surgical anesthesia certainly suggest that the element of asphyxia must be a not inconsiderable factor at the outset. However, with the use of a towel folded so as to admit air rather freely this element can be reduced to a minimum. Once the animal was asleep the ether was administered by continuous insufflation, the tube being inserted well into the trachea. Such a method is of distinct value when procedures are contemplated that interfere in any way with respiration.

For this reason it was selected in our studies on the hypophysis and has been continued into the present series. Macleod (10) used oxygen insufflation in his earlier studies but he has come to discard entirely this principle in his later investigations. He feels that the possible alterations in the reaction of blood may introduce a larger source of error than the one corrected. In other words we may have an over-correction which is more confusing than a slight under-correction.

Samples of blood were drawn from the external jugular vein into oxalate crystals and the sugar was estimated according to the Benedict colorimetric method as modified by Meyers and Bailey (11). The time of the anesthesia was usually three hours with collection of samples just before ether, just after the establishment of insufflation and at one, two and three hour intervals.

The adrenalin was estimated according to method proposed by Folin, Cannon and Denis (12). The animals were fed a mixed diet containing a small amount of meat and an excess of carbohydrates. This diet has been shown by Ross and Hawk (13) and more recently by Ross and McGuigan (14) to be more conducive to a stable blood sugar level than one rich in protein.

TABLE 1

Effect of ether administered by continuous insufflation on blood sugar of normal dogs

ANIMAL	BEFORE ETHER	AFTER ETHER			
		15 minutes	1 hour	2 hours	3 hours
1*	0.142	0.136			
	0.143	0.143	0.165		
2	0.102	0.184	0.191	0.213	
	0.101	0.183	0.200	0.209	
3	0.121	0.163	0.163		0.186
	0.120	0.167	0.163		0.182
4	0.114	0.206	0.196		
	0.116	0.180	0.192		0.192
5	0.105	0.116	0.178	0.220	0.220
	0.107	0.117	0.165	0.212	0.228
6	0.087	0.110	0.153	0.150	0.187
	0.093	0.113	0.156	0.157	0.165
7	0.086	0.117	0.195	0.215	0.203
	0.092	0.117	0.167	0.215	0.184
8	0.098	0.119	0.125	0.159	0.161
	0.099	0.125	0.124		0.146
9	0.101	0.146	0.175	0.216	0.237
	0.101	0.143	0.165	0.220	0.233
10	0.092	0.114	0.140	0.155	0.162
	0.092	0.093	0.136	0.157	0.160

* Dog fed same day, all others on day preceding.

Normal animals. Examination of table 1 shows that despite the precautions outlined above, the sugar level tends to a constantly higher point reaching this at the end of the second hour. The third hour of etherization does not raise the level appreciably.

In four of the ten animals a concentration of 0.2 per cent or more was reached, a quantity which Macleod (15) has designated as a hyperglycemia.

Bilateral splanchnectomy, In animals with both splanchnic nerves (table 2) cut, the rise is confined to the early stages of anesthesia (fifteen minute period) and has worn off at end of two hours. Similar findings have been reported when the hypophysis was stimulated following complete section of splanchnics. We certainly can conclude that the mechanism of the persistent rise in glycemia is mediated through the splanchnic nerves. However, a transitory rise in the early stages may occur without its mediation. Nishi (16) has reported similar findings in studying diuretin hyperglycemia.

TABLE 2

Effect of ether administered by insufflation on doubly splanchnectomized dogs

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER			
			15 minutes	1 hour	2 hours	3 hours
11	7	0.102	0.139		0.101	0.109
		0.099	0.140		0.102	0.105
12	12	0.098	0.116		0.096	0.093
		0.099	0.121		0.097	0.089
13	90	0.102	0.145		0.117	0.115
		0.103	0.141		0.111	0.110
14	14	0.074	0.081		0.082	0.064
		0.075	0.082		0.080	0.064

Unilateral splanchnectomy. Section of splanchnics on one side (table 3) does not abolish the rise in sugar. The level which is attained on the whole appears to be less than in the normals. In only one case (no. 22) was a concentration of 0.2 per cent reached. Animals 23, 24 and 27 are noticeably lower than normal. The findings in no. 21 may be disregarded since the animal was shown to have a localized peritoneal abscess despite which, however, he clinically was enjoying good health. All others were in good shape and had apparently completely recovered from the effects of the operation as shown by mental attitude, healing of abdominal incision and appetite.

TABLE 3

Effect of ether on blood sugar in animals with splanchnic nerves cut on one side

ANIMAL	DAYS SINCE OPERATION	SIDE OF OPERATION	BEFORE ETHER	AFTER ETHER		
				15 minutes	2 hours	3 hours
15	18	Left	0.091 0.090	0.127 0.120	0.116 0.120	0.135 0.144
16	8	Left	0.084 0.082	0.101 0.105	0.122 0.113	0.155 0.148
17	18	Left	0.097 0.094	0.134 0.120	0.190 0.192	0.181 0.197
18	16	Right	0.083 0.087	0.098 0.104	0.155 0.146	0.160 0.158
19	14	Right	0.074 0.077	0.145 0.144	0.176 0.143	0.152 0.146
20	13	Right	0.092 0.099	0.093 0.093	0.122 0.121	0.171 0.176
21*	11	Left	0.075 0.080	0.105 0.105	0.105 0.099	0.110 0.100
22	11	Right	0.077 0.079	0.110	0.182 0.172	0.202 0.184
23	9	Right	0.077 0.082	0.104 0.109	0.113 0.109	0.116 0.106
24	12	Right	0.086 0.086	0.101 0.105	0.120 0.111	0.117 0.110
25	18	Left	0.093 0.088	0.118 0.115	0.128 0.119	0.136 0.131
26	12	Left	0.092 0.095	0.146 0.129	0.135 0.133	0.162 0.147
27	11	Right	0.086 0.091	0.133 0.127	0.103 0.102	0.121 0.118
28	11	Right	0.081 0.080	0.136 0.132	0.132	0.147 0.136

* Abscess walled off by stomach, spleen and parietal peritoneum.

Hawk (2) has called attention to starvation as a predisposing factor to glycosuria. Had our results been in the other direction we should have been inclined to think that the animals had not returned to a normal metabolic level. We may consider that the mechanism has been modified by cutting off one of the final common pathways, a procedure which may possibly result in delimiting the afferent field but more probably exerts its influence on the effector side of the mechanism.

TABLE 4

Effect of ether on blood sugar in animals with denervated hepatic arteries

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER			
			15 minutes	1 hour	2 hours	3 hours
29	17	0.112 0.115	0.145 0.143	0.160 0.154		
30	14	0.136 0.137	0.273 0.273	0.273 0.240		
30*	20	0.107 0.101	0.172 0.168	0.255 0.255	0.317 0.322	
31	10	0.093 0.090	0.162 0.165	0.188 0.188	0.212 0.216	0.258 0.242
31	29		0.116 0.116	0.111 0.114	0.153 0.157	0.156 0.153
32	12	0.096 0.097	0.116 0.116	0.143 0.148	0.200 0.198	
33	12	0.097 0.095	0.136 0.134	0.190 0.178	0.190 0.184	
33	28		0.164 0.164	0.199 0.206	0.206 0.194	0.224 0.218

* Fed on same day.

Certainly the splanchnic nerves appear to possess a quantitative influence and damage to any part of them would appear to have a mathematical influence on the glycemia. So far as our experiments go, we are unable to assign to either the right or the left side a more important valuation. This is in contrast to the findings of Nishi (16) who states that section of left splanchnics in rabbits prevented diuretin hyperglycemia while division of the right side had no effect on its course.

Section of nerves of hepatic pedicle. In the first series of animals only the plexus of nerves attached to the hepatic artery was sectioned. The results of table 4 show that this has not modified the hyperglycemia. The suggestion even arises that the tendency to hyperglycemia is greater than normal.

TABLE 5

Effect of ether on blood sugar in animals with complete denervation of hepatic pedicle

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER		
			15 minutes	2 hours	3 hours
Approximately 40 cc. of blood withdrawn at each sample. Nutritional state normal					
34	17	0.089 0.091	0.110 0.110		0.175 0.172
35	17	0.088 0.083	0.097 0.097		0.148 0.140
36	17	0.078 0.074	0.095 0.100		0.135 0.126
Nutritional state below par since last experiment					
34	20	0.119 0.116	0.191 0.188		0.252 0.242
35	20	0.100 0.095	0.170 0.182		0.211 0.211
36	20	0.091 0.094	0.154 0.150		0.144 0.154
15 to 20 cc. of blood withdrawn at each sample. Nutritional state normal					
34	53	0.093 0.095	0.120 0.114	0.133 0.137	0.133 0.132
35	53	0.073 0.072	0.094 0.092	0.102 0.104	0.127 0.121
36	48	0.083 0.089	0.101 0.094		0.114 0.121

TABLE 5—*Continued*

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER		
			15 minutes	2 hours	3 hours
Two 30 cc. samples withdrawn 3 hours previously, at 20 minute intervals; 10 cc. withdrawn for sugar estimation. Nutritional state normal					
34	59				0.139 0.132
35	59				0.142 0.145
36	59				0.078 0.081
			AFTER 3 HOURS		
			Sample of 10 cc. withdrawn	60 cc. withdrawn and then a second 10 cc. for sugar estimation	
35	73	0.103 0.093	0.127 0.110	0.166 0.168	

Macleod (17) has shown that in denervating the liver he was able to get consistent results only after dividing all the structures of the hepatic pedicle except the portal vein and cauterizing this lightly. Our procedure involved the complete division of all tissues except the artery, vein and common duct and the cauterization of these. In the first three (table 5) the rise in blood sugar is evident and appears to be only slightly less than that occurring in normal animals.

A day was allowed to intervene and the experiment repeated. This group of experiments gave higher results, which we believe are to be attributed to conditions comparable to partial starvation as reported by Hawk (2). Further relatively larger samples (40 cc.) were withdrawn in both of these series. Repetition after feeding the animals for a number of days and withdrawing smaller samples (15 to 20 cc.) of blood gave a decidedly lower level in the next six experiments. Finally the effect of even a small hemorrhage is shown in the case of dog 35; after three hours of anesthesia in which only two samples of 10 cc. had been removed, the level of sugar was 0.127 and 0.110. Sixty cubic centimeters were withdrawn and the glycemia reached a point of 0.166 and 0.168.

King and associates (5) found a normal rise in glycemia after section of hepatic nerves. No mention is made of cauterizing the portal vein. They were also using a meat diet for their animals which, it will be recalled, is conducive to hyperglycemia. We may conclude that these animals show a marked glycemic response to hemorrhage even in small amounts but the response to ether is less than in unoperated animals.

Eck fistula. King and associates (5) found that a hyperglycemia occurred in dogs upon which an Eck fistula had been established. The level of this was slightly over 0.1 per cent for two hours of ether administration (four experiments).

Table 6 shows that in a period of three hours the rise may be quite as high if not higher than normal, though the effect may not be so apparent in the first fifteen minutes. It is highly probable that the operation has not modified the course of events but on account of the smaller blood supply to the liver a longer time is required in which to transfer the excess sugar to the circulation.

TABLE 6

Effect of ether on blood sugar in dogs with Eck fistula

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER	
			15 minutes	3 hours
37	30	0.094 0.092	0.095 0.097	0.190 0.186
38	28	0.110 0.111	0.125 0.116	0.171 0.178
38	41	0.108 0.107	0.116 0.126	0.241 0.251
39	26	0.102 0.095	0.132 0.131	0.218 0.215

Macleod has reported that the acute effect of Eck fistula operation is an acceleration of the glycogenolytic processes. McGuigan and Ross (18) studied same question reporting a hyperglycemia. However our experiments are not directed toward this end. We were endeavoring to see how a liver whose circulation was thus crippled would respond to ether stimulation, and our data show that a hyperglycemia of the same or even greater magnitude than normal may be attained.

Ligation of hepatic artery in Eck fistula animals. Since the animals were available and we desired to get some knowledge of the efficiency of the collateral circulation, the hepatic artery was ligated and the blood sugar followed until death. McGuigan and Ross (18) have reviewed the literature of this subject and reported findings similar to those recorded below. Since their ligations of two vessels were performed within a short time of each other it seemed worth while to record present data obtained by succeeding ligations. The protocols are attached.

Effect of ligation of hepatic artery in dogs with Eck fistula

Dog. 37. February 3, 1918

Time since operation	49 days
Blood sugar estimations before anesthetic	0.104 0.091
After anesthetic just before ligation	0.191 0.200
Four hours after ligation	0.032 0.032
Five hours and ten minutes after ligation just before death	0.018 0.018

Dog 38. February 20, 1918

Time since operation	68 days
Estimations of blood sugar before anesthetic	0.093 0.085
After ether just before ligation of artery	0.128 0.120
One and one-half hour after ligation	0.128 0.130
Two and one-half hours after ligation	0.103 0.106
Five hours	0.093 0.097
Twenty-four hours	0.095 0.097
Thirty hours, just at time of death	0.043 0.039

Dog 39. February 20, 1918

Time since operation	66 days
Blood sugar estimations before anesthetic	0.088 0.088
After anesthetic, just before ligation of hepatic artery	0.124 0.126
One and one-half hour after ligation of artery	0.110 0.107
Four hours after ligation	0.091 0.092
Six and one-half hours after ligation	0.061 0.062

Animal died during night

The first animal (dog 37) died rather quickly (five hours and ten minutes). The blood sugar promptly fell to a level of 0.018.

In dog 38 the hypoglycemia appeared during the last six hours of life; in dog 39 it was definitely present in a sample taken at six and one-half hours. The animal died sometime during the night. It is evident that the collateral circulation was insignificant and that the hypoglycemia as has been previously shown is a part of the terminal syndrome.

Reversed Eck fistula. In these animals the anastomosis between the two veins was performed as usual but the ligature was placed about the vena cava thus directing all the venous blood through the liver.

McGuigan and Ross (18) have reported that this procedure per se does not cause a rise in glycemia.

Our experiments (table 7) show that ether causes a rise comparable in all respects to that found in normal animals.

TABLE 7

Effect of ether on blood sugar in dogs with reversed Eck fistula

ANIMAL	DAYS SINCE OPERATION	BEFORE ETHER	AFTER ETHER	
			15 minutes	3 hours
40	26	0.078	0.117	0.128
		0.079	0.113	0.135
41	27	0.132	0.185	0.207
		0.131	0.182	0.202
42	29	0.094	0.140	0.145
		0.092	0.139	0.142

Adrenalin content in unilaterally splanchnectomized animals. The most mooted question is the part played by the adrenalin in the hyperglycemia. Examination of table 8 shows that in four cases (17, 18, 26, 27) there was no significant difference in the load of the two glands. In four others (16, 19, 20, 23) the control gland had apparently a higher content, in the remaining six the content was less in the control than the experimental gland.

Elliott (19) has shown for cats that ether exhausts the content of adrenalin in a gland which has not been protected by section of its splanchnic nerves. Having in mind this investigation, we hoped to determine whether a relationship existed between the rise in sugar and the degree of exhaustion. A comparison of tables 3 and 8 shows no relationship whatever exists. It is possible that in the dog some of the fibers of the right side may have crossed to the left adrenal. We believe however that our section would have included these. More probably the ether causes an increased production as well as increased partition and it is on this basis that the inconclusive results are to be explained.

They were consequently not extended to a larger series of cases. The partition of adrenalin from the intact glands under ether anesthesia has been abundantly established by Stewart (20) and his associates. Our results are to be taken as further confirmation that ether is modifying the activity of the gland. The question at issue concerns not the partition of the adrenalin but the use of the adrenalin after it enters the blood stream. Is it there because its secretion and the glycogenolytic processes happen to be controlled by two functionally different classes of fibers running in the same nerve which are being commonly stimulated? Is it there to complete the activity of the fibers governing the glycogenolysis?

TABLE 8

Adrenalin load in glands at end of 3 hours of ether insufflation*

ANIMAL	DAYS SINCE SPLANCHNECTOMY OPERATION	EXPERIMENTAL GLAND	CONTROL GLAND	CONTROL / EXPERIMENTAL; EXPRESSED IN PERCENTAGE
				per cent
15	18	0.99 right	0.65 left	65
16	8	0.35 right	0.54 left	154
17	18	0.74 right	0.78 left	105
18	16	1.06 left	1.08 right	101
19	14	0.14 left	0.33 right	235
20	13	0.69 left	0.82 right	118
21	11	0.60 right	0.39 left	65
22	11	1.20 left	1.06 right	88
23	9	0.67 left	0.77 right	115
24	12	1.08 left	0.89 right	82
25	18	0.88 right	0.86 left	97
26	12	1.34 right	1.46 left	109
27	11	0.53 left	0.45 right	84
28	11	0.84 left	0.53 right	63

* Results expressed in milligrams.

Stewart and Rogoff (8) conclude that it is not a necessary factor since the ether rise can occur without its demonstrable presence. Of four of their experiments only two show a gain (0.087 to 0.151 and 0.142 to 0.176) that does not fall within the limit of experimental error. The other two (0.200 to 0.233 and 0.092 to 0.098) are not significant. The time of their anesthetic was fifteen to twenty minutes.

It will be recalled that in our cases, those animals with bilateral splanchnectomy, a similar early (15-minute period) rise was noted. This however passed off at the end of two hours. Such a rise might be very readily explained on basis of asphyxia which unquestionably should be attributed in a great measure to an altered reaction of the blood. We do not feel that their experiments establish the case against the adrenals playing a part in the ether hyperglycemia unless they show that "the persistent long rise" can occur without their mediation. This experimental condition they have not investigated.

DISCUSSION

That the liver is the ultimate effector organ upon which the stimulus falls is certain from the studies of numerous observers who have investigated the nervous hyperglycemias. As to the intrinsic mechanism involved by which these nervous stimuli result in an increased glycogenolysis we have not concerned ourselves. King, Moyle and Haupt (6) in their investigation, using intravenous ether saline solutions, have concluded that the liver cells are the site of the ether action. The hyperglycemia did not occur until surgical anesthesia was produced. The question arises whether the two conditions are identical. It is not at all certain that the ether introduced through the respiratory tract arrives at the liver in the same concentration as it would were it introduced intravenously. It may be more toxic in this latter state. Indeed the absence of hyperglycemia in bilaterally splanchnectomized animals seems to us to constitute final proof that the site of action is not on the liver cells. For these reasons we believe that they have not answered the question in hand but an entirely different one raised by a new experimental procedure.

The extrinsic mechanism in which we are interested involves the splanchnic nerves with their terminations in the liver and adrenals and the discharge of small amounts of adrenalin. The established tonic effect of adrenalin on sympathetic terminations in other sites suggests strongly that it has a value here. The maintenance of a hyperglycemia (of a lower grade, it is true) with the adrenals intact but liver denervated, furnishes to our minds rather definite evidence that the adrenalin is an essential factor. One can object that in such animals the whole abdominal viscera are exposed to vascular alterations. However very extensive changes in circulation through the liver have not been shown to be accompanied by significant variations in glycemia. We must in our thinking push the site of action of these influences beyond a consideration of the quantity of blood delivered passively to the liver, to a consideration of some more active participation on the part of the liver capillaries and cells. It is in controlling this more active participation that we believe the hepatic splanchnic nerves and the adrenalin play their respective parts. Indeed we believe the mechanism to be identical for ether and electrical stimulation of splanchnic nerve, as reported by Macleod (9). They are only two different exciting agents which are capable of throwing into activity the same effectors.

CONCLUSIONS

1. A persistent hyperglycemia occurs in normal dogs under continuous ether insufflation. This reaches a maximum at end of two hours with little change in the third hour.
2. A transient hyperglycemia passing off at the end of two hours occurs in animals with splanchnic nerves sectioned on both sides.
3. A persistent hyperglycemia of a lower grade occurs in animals unilaterally splanchnectomized. There is no difference in the value of the nerves of the right and left sides.
4. Denervation of the hepatic artery gives a glycemic rise equal to that of normal dogs.
5. Section of all the nerves in the hepatic pedicle with cauterization of the coats of hepatic artery, portal vein and common duct prevents the sugar rise from reaching the high point found in unoperated animals but does not abolish it.
6. Eck fistula does not prevent but only retards the appearance of hyperglycemia in the systemic veins.
7. Ligation of hepatic artery in Eck fistula animals causes a terminal hypoglycemia.
8. Reversed Eck fistula does not influence course of ether hyperglycemia.
9. The adrenalin content in dogs whose splanchnic nerves have been previously sectioned on one side is unequal in the two glands.
10. The partition of adrenalin under ether is considered of significance and not an incident of splanchnic stimulation.

BIBLIOGRAPHY

(1) **Keeton and Becht**: This Journal, 1916, xxxix, 109.
(2) **Hawk**: Proc. Amer. Physiol. Soc., This Journal, 1904, x, 37.
(3) **Underhill**: Journ. Biol. Chem., 1906, i, 113.
(4) **Seelig**: Arch. f. exper. Path. u. Pharm., lii, 489; Ibid., liv, 207.
(5) **King, Chaffee, Anderson and Redelings**: Johns Hopkins Hosp. Bull., 1911, xxvi, 388.
(6) **King, Moyle and Haupt**: Journ. Exper. Med., 1912, xvi, 178.
(7) **Oppler-Bertrand**: Zeitschr. f. physiol. Chemie, 1910, lxiv, 393.
(8) **Stewart and Rogoff**: This Journal, 1917, xliv, 543.
(9) **Macleod**: Diabetes, 52.
Macleod and Pearce: This Journal, 1912, xxix, 419.
Macleod: This Journal, 1908, xxii, 373.
(10) **Macleod and Pearce**: This Journal, 1915, xxxviii, 415.
(11) **Meyers and Bailey**: Journ. Biol. Chem., 1916, xxiv, 147.
(12) **Folin, Cannon and Denis**: Journ. Biol. Chem., 1913, xiii, 477.
(13) **Ross and Hawk**: Arch. Int. Med., 1911, viii, 39.
(14) **Ross and McGuigan**: Journ. Biol. Chem., 1915, xxii, 407.
(15) **Macleod**: This Journal, 1907, xix, 388.
(16) **Nishi**: Arch. f. exper. Path. u. Pharm., 1909, lxi, 401.
(17) **Macleod and Pearce**: This Journal, 1912, xxix, 419.
(18) **McGuigan and Ross**: This Journal, 1916, xxxix, 480.
(19) **Elliott**: Journ. Physiol., 1912, xliv, 374.
(20) **Stewart and Rogoff**: Journ. Pharm. Exper. Therap., 1916, viii, 479.

STIMULATION BY THE OUTWARD DIFFUSION OF AN ELECTROLYTE FROM THE IRRITABLE TISSUE

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The peculiar physiological behavior of barium among the alkaline earth metals has long been known. Its greater toxicity, compared with the other alkaline earths, has been investigated by many, especially by Overton (1) who showed that a sartorius muscle in 0.7 per cent NaCl plus 1:10,000 $BaCl_2$ loses all its irritability in four and one-half hours. Correspondingly, the antitoxic action of barium toward other salts is much less than that of calcium or strontium.

The power of barium to cause rhythmical contraction of striated muscles was established in 1886 by Ringer (2); this was subsequently investigated more fully by Loeb (3). In this respect especially does barium differ radically from calcium and strontium, for it is well known that the twitches in a skeletal muscle caused, for example, by sodium salts can readily be prevented by the addition of calcium or strontium salts to the stimulating solution.

The rhythmical contractions produced by barium salts can also be prevented by the addition of salts of other metals. Ringer showed this to be true for the chlorides of potassium and calcium. I found that 7 cc. m/8 $BaCl_2$ diluted with 3 cc. m/8 KCl is unable to call forth any rhythmical activity (4) although $\frac{1}{8}$ cc. and even $\frac{1}{16}$ cc. m/8 $BaCl_2$ in 10 cc. m/8 NaCl speedily throws the muscle into violent twitches. The present experiments showed that this antagonism toward barium is also shared by the chlorides of strontium and ammonium, to a lesser extent by lithium chloride and by hydrochloric acid. Sodium chloride and more especially sodium hydroxide favor the stimulating action of barium. A muscle previously treated with 0.7 per cent NaCl for thirty or forty minutes responds more energetically to a dilute solution of barium chloride and with a shorter latent period than a muscle not thus treated. This favorable action of sodium chloride is well shown by bathing the muscle in a 6 per cent cane sugar solution previous to its immersion in the barium salt. One of the gastrocnemius muscles which had remained for twenty minutes in 6 per cent cane sugar solution was placed in 1 cc. m/8 $BaCl_2$ plus 9 cc. 6 per cent cane sugar; after a latent period of eighteen minutes a few twitches occurred. The other gastrocnemius muscle, after having remained in m/8 NaCl for eighteen minutes, on immersing in a solution of 1 cc. m/8 $BaCl_2$ plus 9 cc. m/8 NaCl began to contract rhythmically within two minutes and continued to twitch violently for a long time. This action of the cane sugar is due, as Overton states (5), to the removal of the sodium salts from the muscle for on bathing the sugar-treated muscle for a sufficient length of time with 0.7 per cent NaCl the response to barium is normal. This, however, does not give us reason to state, as Overton (1) seems to do, that barium is not a stimulus for the striated muscle. While it is no doubt true that barium has no stimulating effect on a muscle except in the presence of sodium salts, it is also true that a muscle which has remained for some time in a solution of cane sugar loses its irritability to the faradic current. Such a muscle is no longer a normal muscle. Hence we hold that barium salts have a powerful stimulating effect on striated muscles.

Whether the stimulating action of the barium and the antagonistic action of potassium, calcium and other salts are due to effects upon the muscle, nerve or myoneural junction I did not determine, being unable to procure curare. Edmunds and Roth (6) state that curare does not influence the course of the contraction caused by barium chloride in muscles of fowls and that denervation has no effect either; they therefore conclude that barium acts on the muscle cells directly.

The antagonistic action of a compound is demonstrated not only by the fact that the minimal concentration of the barium chloride necessary for stimulation is increased when the muscle has been previously treated with the antagonistic salt or when this salt is used simultaneously with the barium, but also by the abolishing of the contractions by the counteracting solutions when once the twitches have been established by the barium. This can, in case of the barium twitches, be readily demonstrated by placing the actively contracting muscle in m/8 calcium chloride solution. Wishing to prove this also for potassium chloride, a muscle showing a fair degree of activity in a m/160 $BaCl_2$ solution was transferred to a m/8 KCl. To my surprise, instead of an almost immediate inhibition of the twitches as I had been led to expect from previous experiments with this salt, the muscle was thrown into far greater twitches than it exhibited previous to its immersion in the potassium chloride solution. In figure 1 the contractions from *a* to *b* were produced by the muscle in 1 cc. m/8 $BaCl_2$ plus 9 cc. m/8 NaCl; at *b* this solution was replaced by 2 cc. m/8 KCl plus 8 cc. m/8 NaCl. In the other experiment a pure m/8 KCl solution was used with practically the same initial result. Having made certain that this was no accidental affair, I made experiments with other solutions and found that m/8 sodium chloride had a similar but much more prolonged effect. In fact, when a muscle is left long enough in a barium chloride solution for the twitches to cease, the subsequent bathing of the muscle with m/8 sodium chloride causes energetic contractions. In figure 2 the twitches in the dilute barium chloride had practically ceased at *c;* on the application of m/8 sodium chloride at *d* the muscle is thrown into violent twitches.

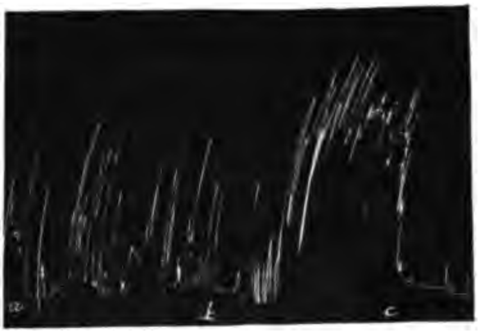


Fig. 1. At *a* the muscle is placed in 1 cc. m/8 $BaCl_2$ + 9 cc. m/8 NaCl; at *b* this is replaced with 2 cc. KCl + 8 cc. NaCl. At *c*, same as at *a*.

In seeking an explanation for the reëstablishing of these twitches when the stimulating agent is replaced with an indifferent or even antagonistic solution, many suggested themselves. At first I was inclined to regard this as an example of the so-called "contact irritability" (7), (8) by which a muscle treated with certain salts is thrown into great activity when the exciting fluid is replaced with almost any other fluid. However, while a muscle showing contact irritability goes into strong rhythmical contractions when it is brought from the sensitizing solution (e.g., sodium citrate) into contact with air, this is not necessarily true for a muscle treated with barium chloride.

Neither can the cause of these contractions in the sodium chloride solution be sought in the mechanical stimulation brought about by the removing of the barium chloride and its replacement with a sodium chloride solution, for the mere removal and return of the barium solution in which the muscle has almost ceased to contract does not reëstablish the twitches. Moreover, experiments to be related presently speak against the view that mechanical stimulation has anything to do with this phenomenon.

Seeing that we are replacing a bivalent kation, Ba, with a univalent kation, Na or K, it might be surmised that this may be the cause of the stimulation. However, a few experiments made with calcium chloride render this very unlikely. In a number of these experiments the change from barium to calcium chloride or magnesium chloride was followed by a slight increase in the twitches, or at least a far better continuation of the twitches than occurs when the muscle is merely taken from the barium chloride and left in the air. Of course, this increase in the contractions is limited as might be expected from the marked antagonism existing between barium and calcium in this matter. And again, in favorable instances the replacement of the barium chloride with 6 per cent cane sugar solution has an effect similar to that shown in figure 2, where the sodium chloride is substituted for the barium. But in the case of cane sugar, as with the calcium chloride, the resulting twitches are of shorter duration, no doubt due to the lack of sufficient sodium salts. As the substitution of either the univalent sodium or the bivalent calcium or even a non-electrolyte for the barium brings about the same qualitative results, these results cannot be attributed to the electric charges of the substances concerned.

Neither is it likely that the cessation of the twitches in the barium chloride is due to the establishment of a physiological balance between the sodium and barium in the muscle and that therefore the influx of sodium, upsetting this balance, causes anew the stimulation of the muscle. When a muscle which has ceased to contract in a solution composed of 10 cc. m/8 NaCl plus $\frac{1}{8}$ cc. m/8 $BaCl_2$ is bathed with 10 cc. m/4 NaCl plus $\frac{1}{8}$ cc. m/4 $BaCl_2$ solution the twitches begin once more, although the proportion of sodium to barium has not been altered. Let us suppose that the original stimulating solution is composed of 9.5 cc. m/8 NaCl plus 0.5 cc. $BaCl_2$; in this the twitches will finally cease. Now it is immaterial whether we substitute 10 cc. m/8 NaCl, 5 cc. m/8 NaCl plus 5 cc. 6 per cent cane sugar, 10 cc. NaCl plus $\frac{1}{16}$ cc. $BaCl_2$ or 8 cc. NaCl plus 2 cc. $BaCl_2$; in every instance the muscle shows increased activity. While it is conceivable that a physiological balance of sodium and barium might result in an inhibition of twitches, it is difficult to see how the upsetting of this equilibrium either by an increase or a decrease in the barium or in the sodium should in all cases act as a stimulation. To do so would be to suppose that barium in a certain concentration has an inhibiting influence over the stimulating action of sodium chloride and that the sodium in this same concentration prevented the barium from stimulating. For such mutually inhibiting qualities we have found no evidence.

That the barium chloride has made the muscle more irritable toward the sodium chloride is hardly likely to be the cause of the stimulation when the barium chloride is replaced with sodium chloride for, as I have already stated, the stimulation takes place whether we introduce a sodium chloride of greater or of lesser concentration than that with which the stimulating barium chloride solution was diluted. And the fact that in favorable cases a sugar or a potassium chloride solution is able to evoke the contractions after the muscle has been treated with a barium salt speaks against the view that increased irritability toward sodium is the cause of these renewed twitches.

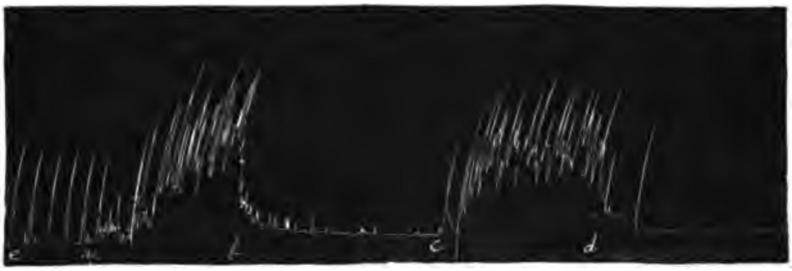


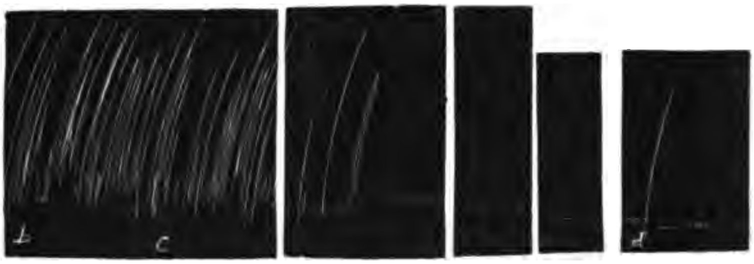
Fig. 2. From *e* to *a* the muscle is placed in 0.5 cc. m/8 $BaCl_2$ + 9.5 cc. m/8 NaCl. At *a* and *c* m/8 NaCl is applied; at *b* and *d* the m/8 NaCl is replaced with 0.5 cc. $BaCl_2$ + 9.5 cc. NaCl.

It is therefore not the upsetting of a physiological balance of barium and sodium nor the passage of sodium into or out of the muscle that can be regarded as the stimulating agency. The results of the experiments related seem to be explicable only on the theory that the actual passage of the barium salt into or out of the muscle and not its mere presence in the muscle stimulates. To prove this four or five experiments were made.

1. When a muscle has become quiet in a dilute barium chloride solution, the immersing of the muscle in a sodium chloride solution reinstates the twitches. If now, while the twitches thus caused are at their highest, the sodium chloride solution is removed and the barium chloride is replaced, the twitches cease very speedily. In figure 2 the twitches

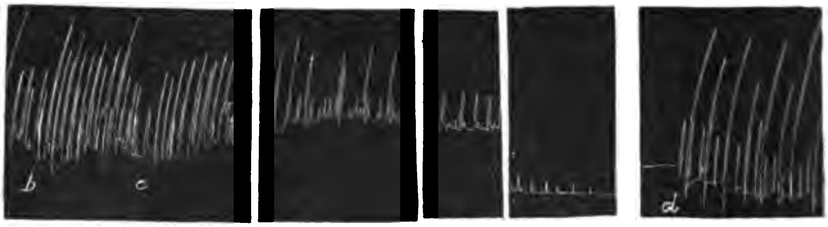
following *a* and *c* are due to the substitution of 10 cc. m/8 NaCl for 0.5 cc. m/8 $BaCl_2$ plus 9.5 m/8 NaCl. When the twitches were well marked, at *b* and *d*, the sodium chloride was removed and 0.5 cc. $BaCl_2$ plus 9.5 cc. NaCl substituted. It will be seen that the twitches cease almost immediately.

2. The two gastrocnemius muscles were prepared and placed in 0.5 cc. $BaCl_2$ plus 9.5 cc. NaCl; in figures 3 and 4 the initial twitches are those caused by this solution. At *c*, in both figures, the barium chloride is removed (after having acted for thirty minutes) and replaced with sodium chloride. In figure 3 this sodium chloride is not renewed but in figure 4 a constant renewal takes place. The parts of the two curves numbered from *1* to *5* correspond in time. It will be seen that although the muscle in figure 3 was originally more active than its mate, fourteen minutes after the application of the sodium chloride



1 2 3 4 5

Fig. 3. From *b* to *c* the muscle is placed in 0.5 cc. m/8 $BaCl_2$ + 9 5 cc. m 8 NaCl. At *c* this is replaced with 10 cc. m/8 NaCl in which the muscle remains till *d* when the original solution is replaced.



1 2 3 4 5

Fig. 4. From *b* to *c* the muscle is placed in 0.5 cc. m/8 $BaCl_2$ + 9.5 cc. m/8 NaCl. At *c* this is replaced with NaCl which is continually renewed. At *d* the NaCl is replaced with 0.5 cc. $BaCl_2$ + 9.5 cc. NaCl.

(in section 2) the twitches cease while they continue for ten minutes longer in figure 4. When the sodium chloride solution is not renewed an equilibrium between the barium salts in the muscle and in the surrounding fluid is soon established; the outward diffusion of the barium salt then ceases and the muscle is no longer stimulated. On the other hand, when the muscle is constantly supplied with fresh sodium chloride solution the diffusion of the barium salt out of the muscle continues for a longer length of time and hence the stimulation is also of greater duration, this ceasing when all the salt has been removed.

3. This theory of stimulation by the outward diffusion of the barium is further supported by the continuation of the above experiments illustrated in figures 3 and 4. When both muscles had become quiescent in the sodium chloride solution, at *d* figures 3 and 4, this solution is removed and the original solution of 0.5 cc. $BaCl_2$ plus 9.5 cc. NaCl substituted. In figure 3, in which previous to *d* the sodium chloride solution had not been renewed, there is little or no activity. On the other hand, the muscle which had been bathed continually with fresh sodium chloride solution almost immediately goes into strong contractions when treated with the original barium solution at *d*, figure 4. In figure 3, the presence of a considerable amount of barium chloride in the muscle prevents the rapid and extensive passage of this salt into the muscle and consequently this muscle is not stimulated to any great extent. But when by the proper washing with a large volume of sodium chloride solution the barium salt is removed from the muscle (fig. 4), the subsequent application of barium chloride is followed by a quick entrance of this salt into the muscle and, as a result, stimulation occurs.

4. In another experiment the two muscles are placed in the proper concentration of barium chloride. After a certain time this is replaced with a sodium chloride solution. In one muscle, say A, this sodium chloride solution is not renewed and the twitches, as related above, speedily come to a standstill. The other muscle, B, is continually washed with fresh sodium chloride solution and its twitches continue for a much longer length of time than those of A. If now, while muscle B is still actively twitching in the sodium chloride solution, this solution is removed and replaced with the sodium chloride solution in which muscle A has ceased its activity, muscle B also very soon becomes quiet. Evidently a sufficient amount of barium salt had diffused from muscle A into the sodium chloride solution to stop the diffusion of the barium salt from muscle B when it is placed in this solution; as the outward diffusion of barium ceased, the stimulation also ceased.

5. It is of interest to compare the results of the application of dilute barium chloride at *b* and *d* in figure 2 with those obtained at *d* in figure 4. In figure 2 the muscle is energetically twitching in the sodium chloride which has been applied for only about a minute; the barium salt is still actively diffusing from the muscle and has by no means reached the end; the application of the barium chloride stops this outward diffusion, but because of the relatively large amount of barium in the muscle, no very active entrance of barium into the muscle is possible and hence there is no stimulation. But in figure 4 the sodium chloride solution having been frequently renewed has removed all the barium from the muscle and hence immersing the muscle in a dilute barium chloride solution at *d*, causes the passage of barium into the muscle; this results in stimulation.

6. That the twitches ensuing upon the replacement of barium chloride with sodium chloride are in reality due to stimulation by barium chloride is rendered highly likely by the fact that when the solution following the barium is composed of potassium chloride or calcium chloride (diluted with sodium chloride), there is at first an increase in the muscle activity, but this activity ceases in most instances very abruptly because of the great antagonism existing between the barium and the potassium or calcium salts.

On looking through the literature for other instances where not the mere presence of the stimulating agent in the tissue but its actual passage into or out of the tissue stimulates, I was able to find only two or three references to this matter. In 1907 Straube (9) found that muscarin which had already penetrated the heart was without physiological action. This investigator placed the heart in a solution of muscarin; after a certain period of inhibition spontaneous recovery took place. At the time of the resumption of the heart beat, the blood in the heart and extracts made from the heart contained sufficient muscarin to inhibit a freshly excised heart. Straube however does not speak of the removal of the muscarin from the heart.

Neukirch (10) observed that the tonus induced in the musculature of the small intestines by means of pilocarpin was increased when the pilocarpin was removed by bathing the tissue with water. This stimulating action Neukirch attributes to the outward diffusion of the pilocarpin from the tissue, but how this causes stimulation he does not attempt to explain. Somewhat similar effects were noted by Meyer (11) in the action of eucain and suprarenin on the contraction of the musculature of blood vessels. In all of these investigations the stimulating agencies employed were complex organic compounds and the methods of experimentation more or less complicated.

The question of why the static presence of an introduced electrolyte in a muscle does not stimulate while its actively passing into or out of the muscle is accompanied by increased activity is naturally but one phase of the general question of what constitutes stimulation. According to the "membrane theory" of Overton, Hoeber, Lillie and others, an increase in the permeability of the plasma membrane is one of the first links in the chain of events which finally finds expression in the peculiar activity of the tissue. When this increase in permeability is abnormally great the stimulation gives place to toxic action (12). It seems difficult to harmonize this theory with the above described results. Let us suppose a muscle-nerve preparation is placed in 0.5 cc. $BaCl_2$ plus 9.5 cc. NaCl. After a latent period varying from one-half to five minutes, vigorous contractions take place, which cease after a certain length of time. The stimulation producing this activity is attributed to an increase in the permeability of the plasma membrane. The subsequent cessation of activity might be explained by supposing that a lower concentration of the barium salt on the surface of the membrane increases the permeability but that a higher concentration decreases this property. Aside from the fact that at present there is no evidence for this, how can we explain that this quiescent muscle, if placed in a more concentrated barium chloride solution, renews its twitches? And if, according to Lillie, the parallelism existing between the stimulating power and the toxicity of chemical compounds is due to qualitatively similar effects on the permeability of the protoplasmic surface film, the matter is still more complicated. For if a muscle ceases its activity in a dilute barium chloride solution because the concentration of the barium in the muscle is so great as to reverse its usual action on the plasma membrane and thus to decrease its permeability, how then, according to the membrane theory, can the continued presence of the barium in this concentration exert any toxic influence? If it is argued that the continued presence of the barium gradually increases the permeability and thereby destroys the integrity of the cell (for which reason the stimulation and increased activity cease and the cell gradually dies), the only way in which the removal of the barium can renew the stimulation is by restoring the permeability to its normal condition, i.e., by decreasing the permeability. But according to the membrane theory (13), a decrease in permeability causes not stimulation but inhibition.

SUMMARY

1. Barium chloride does not stimulate by its mere presence in the irritable structure but by its active diffusion into or out of the tissue. It is therefore possible to cause cessation of the rhythmical contractions by immersing the muscle in a barium solution which originally caused rhythmical activity.

2. The stimulating action of barium chloride is counteracted by the chloride of potassium, ammonium, calcium and magnesium. Sodium chloride increases the effect of barium salts.

3. Bathing a muscle with 6 per cent cane sugar solution speedily reduces its irritability toward barium salts, due to the removal of sodium salts from the muscle.

BIBLIOGRAPHY

(1) **Overton**: Arch. f. d. gesammt. Physiol., 1904, cv, 176.
(2) **Ringer**: Journ. Physiol., 1886, vii, 291.
(3) **Loeb**: Decennial Publications, Univ. of Chicago, 1902, x, 4.
(4) **Zoethout**: This Journal, 1904, x, 211.
(5) **Overton**: Arch. f. d. gesammt. Physiol., 1902, xcii, 349.
(6) **Edmunds and Roth**: This Journal, 1908, xxiii, 46.
(7) **Loeb**: This Journal, 1901, v, 362.
(8) **Zoethout**: This Journal, 1904, x, 324.
(9) **Straube**: Arch. f. d. gesammt. Physiol., 1907, cxix, 135.
(10) **Neukirch**: Arch. f. d. gesammt. Physiol., 1912, cxlvii, 153.
(11) **Meyer**: Zeitschr. f. Biol., 1908, l, 93.
(12) **Lillie**: This Journal, 1909, xxiv, 459.
(13) **Bayliss**: Science, N. S., 1915, xlii, 509.

OBSERVATIONS ON THE CATALYTIC POWER OF BLOOD AND SOLID TISSUE

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At frequent intervals during the past year there have appeared in the literature publications regarding the substances in the blood and body tissues which have the power of releasing oxygen from hydrogen peroxide. The results published have shown a remarkable uniformity, and from the interpretation placed upon them the reader is led to infer that the activity of these enzymes is responsible for many of the physiological processes of the body.

According to Burge, the variations in the activity of these enzymes explain, among others, these phenomena: *a*, The physiological differences between used and unused muscles (1); *b*, Differences between the ratios of heat production (oxidation) in large and small animals (2); *c*, The loss of weight during starvation (3); *d*, Loss of weight during thyroid feeding (4); *e*, The mechanism of phosphorus poisoning (5); *f*, Certain body changes due to emotions (6); *g*, Mechanism of action of anesthetics (7), (8), (10); *h*, Shock (9); *i*, The specific dynamic action of food in the sense of Rubner (11), (12), (13); *j*, The physiological basis for the therapeutic use of alcohol in diabetes (14); *k*, A therapeutic basis for the use of saccharin as a food.

What seemed *a priori* like an error in reasoning in one of the earlier papers (5) of this series led the writer to repeat a few of the experiments, the recent editorial in one of the widely read medical publications (16) led to the completion of this article and its publication.

The substances releasing oxygen from hydrogen peroxide we will call *catalase*, with the understanding that the term is used in the sense suggested by Kastle (17), and the use of the term in no way commits us to any view regarding the nature of the substances concerned. The usual and probably the correct view is that the catalase is an enzyme widely distributed in nature.

The views regarding the function of catalase in the animal economy are as follows: Loew (18), the pioneer in the field—believes the function of catalase to be the destruction of any peroxides formed during the life of the cell, which by their activity would prove harmful to the organism. This same view was advocated by Hertzlika (19) and Shaffer (20). Batelli and Stern (21) observing that catalase inhibits certain oxidations by ferrous sulphate, believe that the function of catalase is the protection of the body by the prevention of excessive oxidation within the cell. Jolles and Oppenheim (22) see in the catalase the substance which releases oxygen from hemoglobin for use by the cell. The same view was advocated later by Ewald (23). Burge (1) claims that measuring the catalytic power of the tissue of an animal measures the rate of metabolism of the tissue. This view was based upon the observation that, in his determinations, the catalytic power of an active muscle like the heart is greater than the catalytic power of less active muscles like those of the leg; while the latter show greater catalytic power than the still less active muscles of the head. Further (2) starting with the well-known fact that the oxidative power of small animals is greater than the oxidative power of larger ones, he studied the heart muscle of birds of various sizes: goose, pigeon and sparrow, and of mammals of various sizes: dog, rabbit and rat, and found that the content of the heart muscle in catalase bears an inverse relationship to the size of the animal. From these observations he concludes that a measure of the catalytic power is a measure of the metabolic activity of a tissue.

Inability to reproduce the results obtained by Burge in some of his early papers led us to a study of various types of apparatus until we finally adopted one which gives results with errors varying between 0 and 9 per cent for blood. Usually the error is less than 3 per cent. It is impossible from reading Burge's papers most carefully to arrive at any conclusion regarding the number of determinations made on each sample and therefore we have no way of estimating a fact not stated in the text; viz., the percentage of error in the method he employed.

1. Method. Our apparatus has undergone various developments but, as completed, it consists of six gas burettes each of 250 cc. capacity, provided with a side arm 10 cm. above the open end and a glass stop cock at the upper end. These burettes are fastened open end down on a ring stand. The open end is closed with a rubber stopper through which passes the upright limb of a glass T-piece. The ends of the cross bar of the T-tube are fastened to the corresponding ends from the next burette. In this way the six burettes are all connected with one another. Then the open ends of the end T-pieces are connected with each other by rubber tubing, and the tubing is connected with a 4 liter Mariotte bottle full of distilled water saturated with oxygen. By raising this Mariotte bottle all the burettes can be filled by closing the side arms and opening the glass stop cocks at the top. The pressure of gas inside and outside the burettes can also be equalized after the evolution of oxygen has ceased, by raising and lowering the bottle to the proper level. During the release of oxygen the bottle is placed on the table beside the burettes. To the side arm of each burette is attached a piece of rubber tubing about 18 inches long. This is closed by a pinch clamp except during the release of oxygen. The free end of this tubing is slipped over the side arm of a Bunsen gas washing bottle of 125 cc. capacity. When ready for a test 50 cc. of hydrogen peroxide are measured into the Bunsen bottle by a pipette. The blood is added by means of a pipette through the neck of the bottle, and the cork is quickly inserted. The pinch cock between the bottle and the burette is immediately released, all the bottles are placed in a shaker and the latter is run for 10 minutes at the rate of 275 shakes per minute. The evolution of gas is complete in this time and futher shaking adds nothing to the volume of oxygen released. Thus with each sample of blood we can obtain six determinations simultaneously under uniform conditions of temperature, pressure and agitation (see fig. 1). The results obtained in this way are fairly uniform but even under the conditions described there are variations usually between 0 and 3 per cent. In all cases it is desirable to dilute the blood until the total oxygen given off is less than 100 cc., otherwise the peroxide foams, bubbles are carried over into the burettes and the readings are less accurate.

We are aware of the fact that there is an error in the data in this paper because the release of oxygen took place at a variable room temperature instead of at some fixed uniform temperature for all the experiments. We are also fully aware of the fact that the amount of oxygen released by the same blood from different samples of peroxide varies between wide limits and that the amounts released from the same sample varies with age of the sample and with the reaction. In the results which follow in tabular form, no attempt was made to run all the bloods reported in this paper on a single sample of peroxide. All results assembled in a table with the exception of those in table 1 were run on a single sample of peroxide and at as nearly the same time as the nature of the experiment would permit. Hence while the data given in the different tables cannot be compared directly, yet the figures assembled in a single table are in all cases comparable. The nature of the data here did not make absolute quantities necessary for the object was merely to compare the bloods under different conditions without reference to absolute catalytic power. We cannot compare bloods of different animals as regards *absolute* catalytic power, but we can say that $\frac{1}{15}$ cc. of cat's blood usually releases about the same amount of oxygen as $\frac{1}{2}$ cc. of dog's blood from a single sample of hydrogen peroxide.

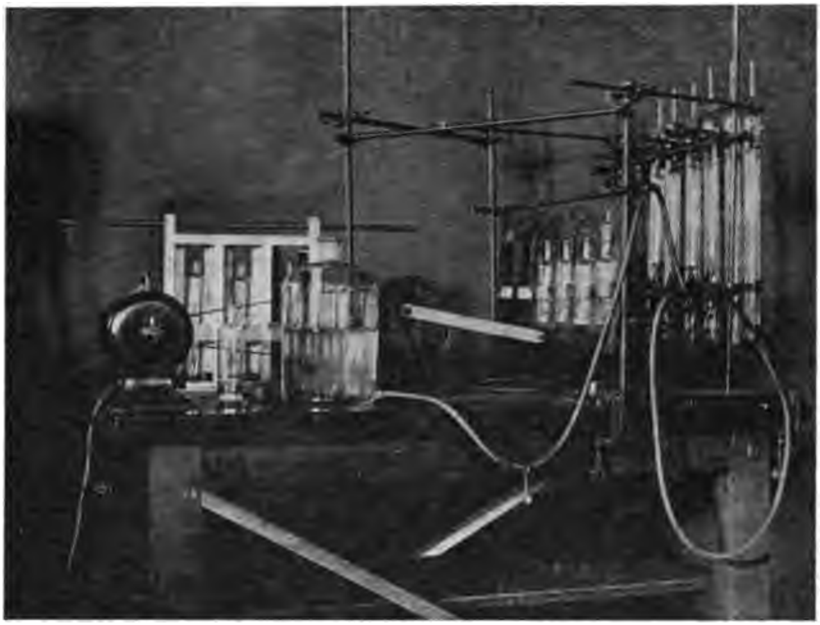

Fig. 1

2. *Normal variations.* An idea of the various readings where repeated determinations were made on the same sample of blood is best secured from table 1.

It is evident from table 1 that the variations where a number of readings are taken are considerable, even where the same samples of blood and hydrogen peroxide are used. For that reason where differences of catalytic power are small in successive samples of blood, too much importance must not be attached to these differences. This applies particularly to much of the past work, from which no idea can be formed from reading the article in regard to the number of determinations made on each sample of blood or tissue.

It is also evident from the data we have given that the amount of catalase present in the blood of animals of the same species varies within wide limits, as well as the fact that variations between species may be even wider. It is, therefore, self-evident that a comparison between the bloods of animals of different species with the idea of obtaining an explanation of the reason why small animals oxidize more rapidly than large ones, is entirely impossible and obviously cannot give any information on the point at issue. Other factors besides mere weight or bulk are certainly at work, and size is only one and relatively an unimportant factor. The catalytic power of the blood of a dog is less than the catalytic power of the blood of a cat of the same weight—a fact which shows clearly the fact that size is only one of probably many factors.

TABLE 1

Variations in oxygen released in simultaneous or successive tests in various animals

ANIMAL	DUPLICATE TESTS USING SAME SAMPLE OF BLOOD ON HYDROGEN PEROXIDE												
	1	2	3	4	5	6	7	8	9	Average	Corrected average at 0°C. 760 mm.	Amount used	Oxygen released by 1 cc. of blood
												cc.	cc.
Dog 1	47	47	47	47	47					47.00	44.14	½	88.28
Dog 2	21	20	22	21	21	21	21			21.00	19.24	½	38.48
Dog 3	13	13	11	12	12					12.20	11.78	½	23.56
Dog 4	79	81	82	80	80	80				80.33	74.53	½	149.06
Dog 5	61	63	64	61	64	64				62.66	59.01	½	118.02
Cat 1	92	93	96	93	96	94				94.00	79.88	$\frac{1}{15}$	1198.20
Cat 2	77	82	82	79	79	80	79	80	81	79.90	67.28	$\frac{1}{15}$	1019.20
Horse 1	67	67	66							66.60	64.89	$\frac{1}{20}$	1297.80
Sheep 1	19	19	19.5							19.00	18.90	$\frac{1}{10}$	189.00
Goat 1	100	101	94	91	93	94	98	101	93	96.10	88.99	½	177.98
Man 1	73	74	73							73.66	62.60	$\frac{1}{15}$	939.00
Man 2	56	54	57	57	55	54	54	57		55.50	50.85	$\frac{1}{15}$	762.60

3. Effect of thyroid feeding. We were, unfortunately from the standpoint of theory, not able to confirm the findings of Burge (5) regarding the results in thyroid feeding. On this part of the subject we carried out three series of experiments; but since the work in the first two series gave such unsatisfactory results—because of wide discrepancies in reading on duplicate tests—we disregarded the results and report here only the data from a satisfactory series of three healthy animals.

The cats were under observation for a period of seven days before thyroid was administered. During this period the heart rate was taken daily and a count of red cells was made. On the seventh day the cats were bled from the jugulars without ether, and then about 15 grams of dry thyroid was administered with a stomach tube and was followed by 75 cc. of whole milk before the tube was removed from the esophagus. The administration of thyroid and of milk was repeated daily during the thyroid period, as well as the taking of the heart rate and the enumeration of erythrocytes. In addition all the cats were fed freely on milk and meat of which they took but little.

TABLE 2

Effect of thyroid feeding

ANIMAL	BEFORE THYROID				AFTER THYROID				Total fed
	Weight	Average heart rate	Blood count (reds)	O_2	Weight	Average heart rate	Blood count (reds)	O_2	
				cc.				*cc.*	*grams*
Cat 23	6½	158.0*	8,460,000*	95.8	6	185.0†	9,495,000†	84.6	90
Cat 24	8¼	148.0*	7,930,000*	81.7	7¼	222.0‡	8,010,000‡	76.9	105
Cat 25	5⅛	183.0*	12,090,000*	169.9	5	186.0‡	9,060,000‡	102.5	105
Average		163.6	9,493,000	119.1		197.6	8,855,000	88.0	

* Average of 7 observations on successive days before thyroid feeding.
† Average of 5 observations on successive days during thyroid feeding.
‡ Average of 6 observations on successive days during thyroid feeding.

For the sake of brevity the results of the series have been condensed into table 2.

As can be seen from the table, all the animals lost weight in spite of the forced feeding; all had an increased heart rate, very slight in no. 25 but marked in the other two; all showed a *decrease* in the power of the blood to release oxygen from hydrogen peroxide, instead of an *increase* reported by Burge (4). In addition notes made at the time the experiments were carried out show that the animals suffered from a severe diarrhea. Thus in every case where the cat is fed thyroid up to the point of the appearance of toxic symptoms, tachycardia, diarrhea and loss of weight, the amount of oxygen released by the blood is in every case less than normal. In our series the average amount released by $\frac{1}{15}$ cc. of blood was 119.1 cc. After thyroid feeding for five to six days the average amount released was 88.0 cc.

We are dealing here with a question of fact, and in favor of our results as compared with those reported by Burge we wish to point out possible errors which we avoided. First, our results are the results of averages, not single determinations. How many determinations he made on each blood cannot be determined from his articles. Ours are the average of nine determinations in each case. Second, in our series each cat constitutes his own control, a much better arrangement because of the wide variation between different animals in the same species. Third, the author referred to could not be certain in regard to the amount of thyroid the animals received for they took the substance mixed in their food. That the author experienced difficulty in getting the animals to eat thyroid is evident from his paper. That our cats received the amounts recorded is certain, that they retained it for at least thirty minutes was covered by continued observation after the drug was administered, that it was absorbed is evident from the toxic symptoms noted above. Fourth, Burge made no attempt to maintain the water content of the blood and thus did not avoid the possible complication of concentration of the blood due to a loss of water by normal excretion and decreased ingestion, or from increased elimination from the diarrhea, if his cats got enough thyroid to manifest toxic symptoms. This factor is important because the loss of water leads to concentration of the form elements, and to an enormous error; for as is known from earlier work (22) and confirmed by data in table 3, the catalases of blood are found in the form element, not in the fluid. We avoided this error by the fact that we administered 75 cc. of whole milk with the thyroid, and we believe this amount to be adequate to maintain the water balance in the blood of cats as large as the ones we employed in our experiments.

Further, it is interesting to note that not only is the amount of oxygen released by the blood reduced by thyroid feeding, but the number of corpuscles required to release a cubic centimeter of oxygen is increased and hence the amount of oxygen released by each corpuscle is reduced. This fact is brought out from the results recorded in the table just mentioned:

Number of corpuscles required to release 1 cc. of oxygen

	BEFORE THYROID	AFTER THYROID
Cat 23	5,887	7,444
Cat 24	6,510	7,100
Cat 25	4,744	5,892

It is possible to estimate the number of cubic centimeters of gas released by 1 corpuscle:

	BEFORE THYROID	AFTER THYROID
	cc.	cc.
Cat 23	0.000169	0.000134
Cat 24	0.000153	0.000140
Cat 25	0.000210	0.000169

From these facts it is clear that not only does thyroid feeding reduce the catalytic power of the blood as a whole, but it also reduces the amount of oxygen released from hydrogen peroxide by each individual corpuscle, in every case. The data here given do not include the leukocytes or platelets. Therefore the loss of weight from thyroid feeding cannot be due to an increased catalase content of blood, for on more accurate measurement *the catalase content of the blood is not increased but is decreased by feeding thyroid.*

Having studied the effect of thyroid feeding we considered it wise to study some of the simple facts regarding the catalytic power of the blood of animals. We selected cats because of the ease with which they could be secured and because of the high catalytic power of the blood. The blood was drawn under light ether anesthesia and thoroughly defibrinated by whipping. All the samples in the series recorded in tables 3 and 4 were run on the same hydrogen peroxide, and the series for each cat was completed on the same day the blood was drawn.

4. The relation of the catalase to the corpuscle. In table 3 we report the results from a series of eight healthy, normal cats in whose blood we studied the catalytic power of whole blood, serum, washed corpuscles and of blood saturated with carbon monoxide (illuminating gas).

This table emphasizes the fact already mentioned in the discussion of table 1; viz: the variation between two animals of the same species

TABLE 3

Effect of washing corpuscles, and of saturation of blood with carbon monoxide

ANIMAL	WHOLE BLOOD	WASHED CORPUSCLES	SERUM	WHOLE BLOOD SATURATED WITH C = O
Cat 1	25.68	26.76	0	23.06
Cat 2	26.72	23.92	0	23.36
Cat 3	26.07	23.43	0	26.07
Cat 4	14.22	12.39	0	12.08
Cat 5	13.65	12.55	0	13.66
Cat 6	16.22	12.27	0	12.89
Cat 7	16.26	12.48	0	15.07
Cat 8	19.75	17.50		18.81
Average	19.82	17.66	0	18.05

In each case 1 cc. of blood was diluted to 10 cc. with 0.9 per cent NaCl, and 1 cc. of the mixture (hence $\frac{1}{10}$ cc. of blood) was used as a test.

is enormous. Thus cat 2 released twice as much oxygen from the same hydrogen peroxide as cat 5; hence the variations amounted to 100 per cent between animals. Yet so far as we could determine both cats were nearly equally active or rather inactive as the readings were taken in the summer. Washing the corpuscles reduces the amount of oxygen released by about 10.8 per cent. We do not believe that this decrease is of much significance; probably the loss of catalytic power is due to loss of corpuscles during the process of washing. That there must be some loss is almost inevitable. That centrifugation is not the cause of destruction is shown by the fact that if blood is merely centrifugated and then shaken up without withdrawal of the supernatant fluid, the readings from the normal blood and centrifugated blood check within the limits of error:

NORMAL BLOOD	CENTRIFUGATED BLOOD	WASHED BLOOD
79.88	79.34	74.29
90.56	90.65	79.09
79.90	81.40	74.00

Of course these experiments do not eliminate a possible "complementing" action between serum and corpuscle.

We are therefore justified in the conclusion that centrifugating the blood does not impair the catalytic power. The third point brought out is the fact that the catalytic power of the blood is confined to the corpuscle, for in none of these cases did the serum release oxygen from hydrogen peroxide. In other experiments serum up to 10 cc. was added to hydrogen peroxide but in no case was more than 2 cc. of O_2 released in ten minutes and in this case considerable red discoloration was evident. Thus the conclusion is warranted that the catalytic power of the blood is resident in the corpuscles and not in the serum. Fourth, saturation of the hemoglobin of the blood with carbon monoxide by exposing the diluted blood to illuminating gas reduces the catalytic power by 8.9 per cent in the series of experiments quoted. In cats 3 and 5 reported in the table release of oxygen by the blood was the same no matter whether the hemoglobin was saturated with oxygen or with carbon monoxide. We have many other cases in agreement with these examples:

	BEFORE	AFTER
Dog	42.07	40.71
	24.45	25.86
	28.10	28.10
Cat	90.55*	92.85*
	67.78*	67.00*
	95.83*	110.60*
	81.67*	94.54*

* This hydrogen peroxide was a different sample from that used in table 3.

Thus the average result from the total of 12 cats tested, in which the blood was saturated with gas after leaving the body the releasing power before saturation was 41.18 cc. and after gas 42.45 cc. of oxygen. Thus it is evident that while in our short series of cases saturation of blood with gas after it leaves the body reduces the catalytic power, this reduction is not evident in a larger series. In the series of three dogs cited the catalytic power before C = O saturation was 31.54, after saturation it was 31.54. These results are in accord with those of Jolles and Oppenheim (22), and opposed to those of Winternitz and Melloy (24). In all probability in the latter's case the change is due not to the direct action of the C = O, as they have assumed, but to some effect of gas upon the body other than the saturation of the hemoglobin with carbon monoxide. Fifth, the table shows the wide variation in the blood from apparently normal cats, there being 100 per cent variation between the highest and the lowest although the animals all seemed equally active and normal. The sluggish animals *did not* show a low, nor the active animals a high oxidase content as is claimed by Burge (13).

Further, dog 4 of table 5 was one of the most active dogs studied, yet his catalytic power was far below the average normal for all the dogs studied. There is absolutely no doubt in the mind of the author that there is *no relation* between the catalase content of the blood and the activity of the animal. The determinating factor as to whether an individual is sluggish or alert would seem to depend not so much on the catalase content of the blood as upon the nervous system.

TABLE 4

Effect of laking agents

	WATER LAKING	FREEZING AND THAWING	SAPONIN		ETHER		CHLOROFORM		ACETIC ACID		NaOH		NORMAL
			Laked	Control	Laked	Control	Laked	Control	Laked	Control	Laked	Control	
1	9.06	5.10	8.78	21.20	18.51	23.43			1.88	21.54	1.56	30.29	25.68
2	10.56	2.90	9.50	24.11	15.23	25.57	9.34	24.85	0.46	24.85	0.61	70.07	26.72
3	17.00	10.39	12.28	25.98	11.62	24.18	12.18	25.50	0.00	23.89	0.28	30.32	26.07
4	5.59	3.02	6.69	15.25	5.32	11.29	2.57	12.21	0.91	14.32	0.46	14.68	14.22
5	3.96	4.70	4.88	12.54	3.32	10.60	1.47	9.77	0.55	11.62	0.27	13.65	13.65
6	6.85	4.14	5.40	16.76	3.24	15.86	2.52	17.12	0.54	14.82	0.00	17.12	16.22
7	5.96	2.31	5.96	14.42	7.39	15.72	7.39	15.07	2.77	17.57	0.00	16.26	16.26
8	10.63	5.73	9.40	18.81	11.58	18.81	10.16	19.38	4.51	19.75	0.00	25.40	19.75
Av....	8.70	4.37	7.86	18.63	9.53	18.18	6.52	17.70	1.45	18.54	0.39	27.22	19.82

Each test was made by adding 1 cc. of a mixture of laked blood 1: 10 to 50 cc. of H_2O_2. Thus the figures represent the oxygen released from 50 cc. of H_2O_2 by $\frac{1}{10}$ cc. of blood. Isotonicity was secured in the case of water laking before testing the catalytic power. The control was made by adding the laking agent to the H_2O_2 before the blood was added.

The next step in the subject was the determination as to what portion of the corpuscle is responsible for the release of oxygen from hydrogen peroxide. Upon this point information was obtained by laking experiments. Various forms of laking were employed as is shown in table 4. This table contains data from the same animals, made at the same time as the results cited in table 3.

It is evident from the table that laked blood does not release as much oxygen from the peroxide as does normal blood. Of the agencies employed, ether was least effective, and NaOH most effective in reducing the catalytic power. None of the substances employed modified the amount released to any very great degree as is shown by the comparison of the control with the normal amount released, except in the case of NaOH. This substance shows a favoring action, undoubtedly by the neutralization of the acid present in the U. S. P. preparation of hydrogen peroxide on the market. It is probable, judging from the colors produced, that the varying reduction of catalase content of the laked blood depends upon the degree of laking rather than upon fundamental differences in the processes of laking. Laking by water and by freezing and thawing are without doubt mechanical, yet these produce marked reductions in the catalytic power. If the presence of enzymes is the only factor concerned in the release of oxygen, it is strange that any process as mild as the mere introduction of water, or simply raising and lowering the temperature between 0°C. and 23°C. should produce such a marked reduction in the activity of the enzyme, for most enzymes withstand the action of water and wide ranges in temperature and readily withstand temperatures around 0°C. Thus it is evident that for normal catalytic activity the presence of the uninjured corpuscular membrane is essential. Whether the loss of catalytic activity is due to the destruction of the enzyme or to the loss of surface of the corpuscle is not clear. It has been impossible up to the present time for us to recover the enzyme, which is stated by Senter (25) to be in combination with the stroma. The laked blood—thus containing the mixture of hemoglobin and stroma—shows very little catalytic action and the addition of even large amounts of stroma—from as much as 10 cc. of blood for a single test—releases very little oxygen, 2 cc. Thus with the laking of the corpuscle the enzyme disappears from the blood and is lost, either because of destruction of the enzyme or because of a loss of surface.

5. *Effect of anesthetics.* Regarding the effect of anesthetics on the catalase content of blood, Burge concludes that the catalase content of the blood of animals is markedly reduced during the induction of anesthesia, and increased to above normal during recovery. The figures published were as follows:

	BEFORE	FOURTH STAGE	DECREASE	AFTER	INCREASE
			per cent		*per cent*
Dog 1	70	48	32	75	56
Dog 2	62	42	29	59	40
Cat 1	875	600	31		

One fundamental error in this work is self-evident: The normal was taken on animals so under the influence of morphine and ether that the insertion of a tracheal cannula was possible—an error inexcusable when the explanation of anesthesia is under consideration. Since animals were being studied in this laboratory under morphine and ether for another problem, it was considered advisable to subject the work on anesthetics to further tests, for this problem is of fundamental importance. The results are to be seen in table 5 where the data from ten experiments on six animals have been assembled.

As can be seen from the table the normal variation observed between individuals of the same species is very wide. The greatest catalytic activity observed was in dog 5, experiment A, whose blood released 66.22 cc. of oxygen. The lowest was dog 4, experiment B, whose blood released only 5.96 cc. of oxygen. Yet both animals were equally healthy and of the two dogs no. 4 was certainly the more lively animal. Thus we cannot credit the statement recently made (13) that it is possible to "pick out dogs with high catalase content on the basis of liveliness." As can be seen from the table, the average normal releasing power of the blood of dogs for oxygen amounts to 34.74 cc. for each ½ cc. of dog blood. After morphine this average is reduced to 33.67 cc. Leaving out experiment C, dog 5, and experiment B, dog 6, in which there was obvious error due to imperfect defibrination of the blood, the average was 35.71 cc. After ether for fifteen minutes it is increased to 37.39 cc., after ether for one hour, or until the death of the animal, 36.92 cc. In only three of ten cases was there a decrease, and in two of these the animal was dead from an overdose of ether. It is, therefore, evident that under ether anesthesia there is no such reduction of catalytic power as is reported by Burge in his articles, (7), (8), (10), in which he states in his conclusion: "The catalase content of the blood decreases during the administration of ether and increases during the recovery from ether." We can offer no suggestions as to why our results differ from those reported by Burge, unless the difficulty lies in the method he employed, which we consider to be extremely inaccurate. However, that does not explain the gradual decrease and increase which he reports. If the errors lay entirely in the method such as we noted when employing his methods, we would expect irregular variations, not the uniform change which he reports.

Further, Burge (14) finds with alcohol an increase in the catalytic power of the blood, and this he believes to be the explanation of the beneficial results of alcohol in diabetes. As is well known, alcohol produces in large doses a condition objectively similar to anesthesia, yet according to Burge's measurements the catalases are increased. Are we to believe that the action of alcohol and ether is fundamentally different? Further his premise that the pathology of diabetes is the failure of the animal organism to oxidize sugar because of decreased catalytic power of the tissue is not yet established.

TABLE 5

Effect of anesthetics

ANIMAL	DATE	CUBIC CENTIMETERS OF OXYGEN RELEASED FROM 50 CC. OF H_2O_2 IN TEN MINUTES BY ½ CC. OF BLOOD, WITH CONSTANT SHAKING								REMARKS
		Normal		After morphine, 30 minutes		After ether 15 minutes		After ether, 1 hr., or death from ether		
		Number of determinations	Average	Number of determinations	Average	Number of determinations	Average	Number of determinations	Average	
Dog 1.....	October 23	5	44.14	5	42.54	5	42.89	5	42.39	Dog dead of ether in 1 hour
Dog 2....	October 25	3	53.96	3	46.22	3	47.96	3	42.07	Dog dead of ether in ½ hour
Dog 3.....	October 26	6	19.24	3	18.32	6	22.60	6	23.74	After observing for 1 hour, dog killed with ether. Blood = 23.85
Dog 4A...	November 6	3	6.53	3	9.58	3	9.89	5	11.78	November 7 = 8.96 cc. November 8 = 9.00 cc.
Dog 4B...	November 9	3	5.96	3	6.27	3	6.92	3	7.21	November 10 = 7.45. November 13 = 4.45
Dog 5A...	November 6	3	66.22	3	69.43	3	70.66	6	74.53	
Dog 5B...	November 8	3	49.79	3	54.74	4	55.86	3	59.47	
Dog 5C...	November 9	3	53.05	3	32.13*	3	61.33	6	59.01	
Dog 6A...	November 8	3	23.82	3	22.27	3	30.42	3	26.77	
Dog 6B...	November 9	3	24.74	3	18.84*	3	25.43	3	22.28	
Average................			34.74		35.71		37.39		36.92	

* Blood not properly defibrinated, numerous clots, and the estimations are too low. These figures are not included in the average of the column.

Naturally since no decrease in catalytic power can be demonstrated, the theory of anesthesia he advocates is untenable.

6. *Catalase of solid tissue.* Our attempts to measure the catalytic power of solid tissue were unsatisfactory. How recent writers secure the uniform results they report is a mystery, to be explained only on the basis of a single test on each sample. If the method of determining catalases is reliable then results obtained by running a series of three to six tests of the same tissue against the same sample of hydrogen peroxide should give uniform results. Further, granting that the above test can be made satisfactorily, in comparing the same tissue from different animals or different tissues from the same animal where the tissues must be prepared separately, what is the criterion by which the fineness of division of the tissues prepared separately can be judged? It is very important that the state of division be the same in the sample to be compared for a finely ground sample will always release more oxygen from hydrogen peroxide than one less finely ground. Blood allowed to clot partially and then whipped so that fine clots are suspended yields less oxygen than blood well defibrinated. (See experiments 5C and 6B, table 5). This introduces the difficulty of preparing tissues from different sources, as for example heart muscle with skeletal muscle, a difficulty so great that a true comparison of the catalytic power of the two tissues has not yet been made.

We wish to present type examples of our results where a number of tests were made on a single carefully prepared tissue run against hydrogen peroxide. These tests involve five tissues only, viz., brain, heart, liver, intestines and skeletal muscles.

Experiments on brain. Young cat. Bled under ether. Aorta and carotid were perfused with warm salt solution. Brain and medulla were removed and ground in mortar for thirty minutes until a uniform, creamy consistency was secured. To 10 grams of brain tissue was added sufficient 0.9 per cent NaCl to make 100 cc. To this emulsion were added 12 large glass beads, and the vessel was shaken until a uniform emulsion was secured. Ten cubic centimeters of this emulsion, and thus 1 gram of brain tissue, were added to 50 cc. of hydrogen peroxide in each bottle, and all tests were run simultaneously for two hours. Readings were made at ten-minute intervals at first and longer intervals later. The results are recorded uncorrected in table 6. The tissue showed a tendency to clump and to rise to the surface during the early part of the shaking. Later the particles remained separate from each other.

Experiments on liver. Dog. Ether anesthesia. The animal was perfused through the aorta with warm salt until the liver was free from blood. The liver was ground in a sausage grinder and then triturated with a mortar and pestle. One gram of the ground tissue was accurately weighed into a watch crystal, washed into the Bunsen bottle by 10 cc. of 0.9 per cent NaCl. Fifty cubic centimeters of hydrogen peroxide were added to each bottle and the tests were shaken as before noted. Readings were made as in the previous experiment. The results are recorded uncorrected in table 7. The tissue floated much of the time.

Experiments on heart tissue. The heart was removed under ether while still beating, and perfused backward through the aorta under about 1 meter pressure with warm salt. The heart beat vigorously during the early part of the perfusing and later was massaged freely until bloodless. The tissue was then ground in the sausage grinder and later triturated with a mortar and pestle until in a very fine state of division. The tissue tests were run as in the case of the liver. Results are recorded in table 8.

TABLE 6

Rate of the release of oxygen by 1 gram brain tissue

TIME	BURETTE					AVERAGE	ERROR
	1	2	3	4	5		
minutes	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
10	76	68	72	72	72	72.0	5.50
20	90	80	84	91	84	85.8	7.25
60	99	97	90	99	95	96.0	6.20
90	104	113	90	107	112	105.2	14.40
120	104	132	90	107	113	109.2	20.90

TABLE 7

Rate of release of oxygen by 1 gram liver tissue

TIME	BURETTE			AVERAGE	ERROR
	1	2	3		
minutes	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
10	203	165	162	176.6	14.8
20	265	219	218	234.0	13.2
30	314	261	265	280.0	12.1
40	378	319	324	340.3	9.00
50	415	361	358	378.0	9.80
70	519	463	478	486.6	6.63

Experiments on intestine. Young cat. Ether anesthesia. Bled from the heart and later perfused with warm salt solution until the tissues were bloodless. The small intestines were removed and thoroughly cleansed of their contents. They were then slit longitudinally and the mucosa and part of the muscularis was scraped off into a mortar. The tissue was then carefully triturated with mortar and pestle. One gram by weight was tested against hydrogen peroxide, as in the previous tests, except that the peroxide had been neutralized with sodium carbonate. The results appear in table 9.

Experiments on skeletal muscle. Muscle was removed from the posterior aspect of the hind legs and lumbar region of the cat from which the intestines had been removed. The muscle was cut finely with the scissors and then ground with an equal amount of clean sand until the tissues were finely subdivided. One gram of this mixture of muscle and sand, thus, 0.5 gram of voluntary muscle was tested against neutralized hydrogen peroxide. The results are shown in table 10. The sand above, added to hydrogen peroxide, released no oxygen in thirty minutes. Therefore, the reaction here recorded is due to the action of substances in the muscle.

TABLE 8

Rate of release of oxygen by 1 gram heart tissue

TIME	BURETTE					AVERAGE	ERROR
	1	2	2	4	5		
minutes	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
10	39	46	39	51	50	45.0	13.3
20	43	48	46	54	55	49.2	12.6
30	44	48	49	55	56	50.4	12.7
40	45	48	51	56	57	51.6	12.7
50	45	48	53	58	60	53.0	15.0

TABLE 9

Rate of release of oxygen by 1 gram intestinal muscle and mucosa

TIME	BURETTE					AVERAGE	ERROR
	1	2	3	4	5		
minutes	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
10	378	339	312	315	391	347.0	12.6
20	452	423	392	405	458	426.0	7.9
30	476	452	420	445	473	452.3	7.1
60	499	488	461	486	498	486.4	4.4

TABLE 10

Rate of release of oxygen by ½ gram of voluntary muscle

TIME	BURETTE					AVERAGE	ERROR
	1	2	3	4	5		
minutes	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
10	126	119	118	124	127	122.8	3.9
20	166	164	191	194	186	180.8	9.2
30	180	175	209	211	205	196.0	10.7
60	196	192	227	232	227	214.8	10.6
90	198	193	232	236	231	218.0	11.4

From these experiments it is evident that when dealing with the solid tissues, even in a finely subdivided state, the evolution of gas is by no means completed in the ten minutes allowed by Burge, or in the fifteen minutes allowed by Alvarez and Starkweather (26), (27), (28), in determining the catalase content of solid tissue. In the case of the brain tissue four of five tests evolved some gas after sixty minutes, and the greatest deviation from the average of five tests varied from 5.5 to 20.9 per cent in the various readings. As can be seen in table 11, in one burette only 51 per cent of the total gas ultimately released was released in the first ten minutes; in another burette under identical conditions 80 per cent of the total gas ultimately released was released in the same time. Taking the average 65.9 per cent was released, and the greatest variation from this average was 14.9 per cent. It is therefore evident that for brain tissue a ten-minute test is absolutely inadequate.

TABLE 11

To show the percentage of the total oxygen released in ten minutes

TISSUE	BURETTE					AVERAGE	DIFFERENCE FROM AVERAGE
	1	2	3	4	5		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Brain	73	51	80	67	64	65.9	14.9
Liver	39	35	33			36.2	3.2
Heart	86	95	73	88	83	84.9	11.9
Intestine	75	67	67	64	78	71.3	7.3
Voluntary muscle	63	61	51	52	55	56.3	5.3

As can be seen from table 12, in one burette only 60 per cent of the total gas ultimately released was released in the first twenty minutes; in another burette under identical conditions 93 per cent of the total gas ultimately released was released in the same time. Taking the average 77 per cent was released, and the greatest variation from this average was 17 per cent. It is therefore evident that for brain tissue a twenty-minute test is absolutely inadequate. In the case of the liver, all the tests were still evolving gas after seventy minutes of shaking. In fact some oxygen was evolved the next morning when the shaker was started after standing untouched all night. The greatest deviation from the average varied between 6.63 and 14.8 per cent. The details of error are to be seen in tables 11 and 12. In the case of the heart three of five tests were evolving gas after forty minutes of shaking and the deviation from the average varied between 12.6 and 15 per cent.

(See tables 11 and 12.) The writers referred to above record no long time experiments and it is thus self-evident that the results recorded by them measure not the total catalytic power of the tissue studied, but only a small and by no means necessarily equal fraction of the catalytic power of each tissue studied. That intestine and voluntary muscle are subject to the same error as the other solid tissues is evident from tables 9, 10, 11 and 12.

We believe that the work we have reported is proof that this method of studying catalase is unreliable, even when the same sample of tissue is used in duplicate tests, because the factor of error is from 5 to 20 per cent if we consider the variations between different tests. The factor of error in the solid tissues when we compare the amount of gas released in ten minutes in any single test with the total gas ultimately set free in the same test may vary anywhere from 3.2 per cent to 14.9 per cent from the average. If the reading is made after twenty minutes the factor of error varies between 2.9 per cent and 17 per cent from the average. The allowance made for either of these factors must be at least *doubled*, if tissue from different animals or different tissues from the same animal but prepared separately, are used, because of the utter impossibility of reducing all the tissue to the same state of subdivision. Thus it is with regret that we are forced to the conclusion that the data on the catalytic power of solid tissues prepared according to the methods employed are worthless, and the theoretical conclusions founded on these data are without the necessary foundations in fact.

TABLE 12

To show the percentage of the total oxygen released in twenty minutes

TISSUE	BURETTE					AVERAGE	DIFFERENCE FROM AVERAGE
	1	2	3	4	5		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Brain	86	60	93	67	63	77.0	17.0
Liver	51	47	45			48.0	3.0
Heart	95	100	86	93	91	92.8	7.2
Intestine	90	86	85	83	91	87.5	3.5
Voluntary muscle	83	85	83	82	80	82.9	2.9

SUMMARY

1. A method which measures the catalytic power of the blood is given accurate to within 5 per cent in most cases.
2. There is 1000 per cent variation in the bloods of different animals (dogs) under identical conditions, and wide variations in the bloods of animals of different species.
3. There is a reduction in the catalytic power of blood during thyroid feeding; this is true not only for the whole blood but for each corpuscle also. Thus increased catalytic power of the blood can not explain the loss of flesh during thyroid feeding, either because of the increased oxidation of material in the blood stream, or because of increased loss of catalase to the blood by the tissues, resulting in over-activity of the autolytic ferments, normally held in check by catalase.
4. The catalytic activity of the blood is confined to the corpuscle.
5. The catalytic power of the corpuscle is not influenced by the oxygen-carrying power of the blood.
6. There is a slight increase in the catalytic activity of blood during ether anesthesia, hence a decrease in the catalytic power of the blood cannot be the mechanism of action of the anesthetics.
7. No method has yet been devised which adequately measures the catalytic power of the solid organ. Oxygen is evolved for at least thirty minutes and the error is from 5 to 20 per cent.

CONCLUSION

Since the catalytic power of the blood varies between such enormously wide limits, 100 per cent to 1000 per cent, in animals of the same species under the same conditions, we do not believe that the catalases can be particularly important and hence do not believe that a study of catalases can possibly explain the mysteries of the processes of oxidation.

(The writer wishes to acknowledge the help of Dr. E. L. Ross in the development of the apparatus, and of Messrs. Eymundson, Greenwood, Hubbard and Schlosser in carrying out the experiments.)

BIBLIOGRAPHY

(1) Burge: This Journal, 1916, xli, 153.
(2) Burge and Neill: This Journal, 1917, xlii, 373.
(3) Burge and Neill: This Journal, 1917, xliii, 58.
(4) Burge, Kennedy and Neill: This Journal, 1917, xliii, 433.
(5) Burge: This Journal, 1917, xliii, 545.
(6) Burge and Burge: This Journal, 1917, xliv, 75.
(7) Burge: This Journal, 1917, xliv, 290.
(8) Burge: This Journal, 1917, xlv, 57.
(9) Burge and Neill: This Journal, 1918, xlv, 286.
(10) Burge, Neill and Ashman: This Journal, 1918, xlv, 388.
(11) Burge, Neill and Ashman: This Journal, 1918, xlv, 500.
(12) Burge and Neill: This Journal, 1918, xlvi, 117.
(13) Burge and Neill: This Journal, 1918, xlvii, 13.
(14) Burge: Science, 1918, xlviii, 327.
(15) Burge: Science, 1918, xlviii, 549.
(16) See editorial, Journ. Amer. Med. Assoc., 1918, lxxi, 1316.
(17) Kastle: Hygienic Lab. Bull. 59, 1909.
(18) Loew: U. S. Dept. Agric. Rept. 68, 1901.
(19) Hertzlika: Quoted from Kastle.
(20) Shaffer: This Journal, 1905, xiv, 299.
(21) Batelli and Stern: Compt. Rend. d. Acad. Sci., 1905, cli, 1044.
(22) Jolles and Oppenheim: Virchow's Arch., 1905, clxxx, 185.
(23) Ewald: Arch. f. d. gesammt. Physiol., 1907, cxvi, 334.
(24) Winternitz and Melloy: Journ. Exper. Med., 1908, x, 759.
(25) Senter: Quoted from Kastle.
(26) Alvarez and Starkweather: This Journal, 1918, xlvi, 186.
(27) Alvarez and Starkweather: This Journal, 1918, xlvii, 60.
(28) Alvarez and Starkweather: This Journal, 1918, xlvii, 67.

THE INFLUENCE OF INTERNAL SECRETIONS ON THE FORMATION OF BILE

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The experiments that form the basis of the present report were undertaken in an effort to determine the effect of certain of the glands of internal secretion upon the output of bile by the hepatic cells. For our study we selected the following gland substances: mammary, orchic, ovarian, pancreatic, splenic, thymic and thyroid. These were all obtained from Armour & Company. To the foregoing list we added solution of adrenalin chloride, prepared by Parke, Davis & Company, and secretin, prepared by the method we have described in connection with other experiments (1).

The procedure was the same in all of the experiments: The animal to be operated upon, usually a dog but in a few cases a cat, was anesthetized by morphine and ether, if a dog, or by ether alone, if a cat; a cannula was inserted in the left carotid artery and connection made with a mercury manometer in order to record blood pressure; another cannula was inserted in the right external jugular vein for the injection of a solution of the gland substance being studied. The abdomen was opened in the linea alba and a third cannula, specially constructed, was tied in the common bile duct; the cystic duct was now clamped or ligated, the edges of the abdominal incision brought together, and a towel wrung out in physiological saline solution at a temperature of 40°C. placed upon the abdomen so as to cover the wound completely. This towel was kept moist. Rectal temperature was taken from the time the anesthetic was started until the end of the experiment. An electrically heated operating table was used.

When all was in readiness an observer, who had no other duty, began to count the drops of bile as they fell from the end of the cannula tied in the common duct. All counts were made for an arbitrary period of twenty minutes. The first count was made before any agent was injected. When this initial count was finished the gland substance to be studied was injected per jugular. In every case, except the experiments with adrenalin, a dose of 10 mgm. of the gland substance per kilogram of body weight of the animal was dissolved in 100 cc. of physiological saline solution, warmed to 37°C. on a water bath, and injected by means of a burette. The counting of the drops of bile was continued for a second twenty-minute period beginning at the moment the injection of the gland substance started. In most instances another twenty-minute count was made, giving an initial count with which comparison could be made and one or two counts after the gland substance had been administered.

No attempt was made to analyze the bile at any time during the course of any experiment so that we are unable to speak with regard to qualitative changes that may have resulted. The number of drops of bile secreted in each period may be regarded as indicative of the activity of the liver cells so far as bile production is concerned during that particular time and it was to this that our inquiry was directed.

The results of all the experiments have been arranged in groups, each group comprising the determinations made with one gland substance, and these are presented in the accompanying tables.

Adrenalin (table 1) produced a decrease during the first twenty minutes in every case. There were three occasions on which it was possible to make a second count after the injection and in two of these the number of drops of bile secreted was still less than during the period of the initial count.

Eight determinations were made with mammary gland substance (table 2); five showed a decrease from the initial count during the first twenty-minute period, two an increase and one no change. Where a second count was made after the injection, which was done in seven of the experiments, no definite trend was observable. It would seem that the effect of mammary gland substance on the secretion of bile is a temporary decrease.

Orchic substance (table 3) caused a lessened secretion of bile during the first twenty minutes after the injection in five out of seven experiments; in four of the five the effect was progressive, a further decrease occurring in the second twenty minutes.

In the five tests made with ovarian substance (table 4) there was a decrease in every instance, averaging 44.59 per cent in the first twenty minutes, and 59.36 per cent in the second twenty minutes.

Pancreas (table 5) was injected into nine dogs and the count of the drops of bile during the succeeding twenty minutes revealed a decrease

TABLE 1

Adrenalin. Dose: 0.1 cc. of a 1: 1,000 solution adrenalin chloride per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
7 (cat)	10	4	−60.00	5	−50.00
13	140	49	−65.00	No count	
27	9	8	−11.11	13	+44.44
47	16	12	−25.00	9	−43.75
60	13	10	−23.07	No count	

TABLE 2

Mammary substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
8	3	2	−33.33	2	− 33.33
11	46	23	−50.00	No count	
16	18	.5	−72.22	2	− 88.88
25	5	5	00.00	15	+200.00
34 (cat)	6	7	+16.66	10	+ 66.66
46	15	12	−20.00	22	+ 46.66
49 (cat)	10	15	+50.00	14	+ 40.00
57	22	13	−40.90	5	− 77.27

TABLE 3

Orchic substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
4	84	45	−46.42	23	−72.61
18	31	3	−90.32	35	+12.90
19	134	15	−88.80	9	−93.20
41	35	12	−65.71	4	−88.57
42	9	8	−11.11	4	−55.55
55	12	25	+108.33	No count	
62	25	35	+40.00	No count	

TABLE 4

Ovarian substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
5	19	18	−5.26	10	−47.36
20	35	8	−77.14	4	−88.57
43	4	1	−75.00	3	−25.00
56	30	27	−10.00	15	−50.00
59	9	4	−55.55	1	−88.88

TABLE 5

Pancreatic substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
1	16	3	−81.25	3	−81.25
14	89	60	−32.58	42	−52.80
22	35	15	−57.11	No count	
23	121	108	−10.74	73	−39.66
30	3	2	−33.33	2	−33.33
38	42	22	−47.61	25	−40.47
48	8	3	−62.50	3	−62.50
50	25	17	−32.00	25	00.00
52	25	12	−52.00	No count	

TABLE 6

Secretin. Dose: 10 mgm. of a dried acid extract per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
9	31	35	+12.90	36	+16.12
10	1	6	+500.00	4	+300.00
15	2	6	+200.00	36	+1700.00
26	1	6	+500.00	1	00.00
35	36	64	+77.77	55	+52.77
36	4	18	+350.00	No count	
54	2	3	+50.00	No count	

TABLE 7

Splenic substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
2	198	198	00.00	207	+4.54
21	89	45	−49.43	30	−66.29
24	4	8	+100.00	6	+50.00
31	207	205	−0.96	No count	
44	36	19	−47.22	20	−44.44
45	6	7	+16.66	6	00.00
61	37	18	−51.35	7	−81.08

TABLE 8

Thymic substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
3	55	18	−67.27	61	+10.90
29 (cat)	42	38	−9.52	No count	
32	37	17	−54.05	13	−64.86
39	45	17	−62.22	31	−31.11
53	31	7	−77.41	2	−93.54

TABLE 9

Thyroid substance. Dose: 10 mgm. per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	1ST COUNT AFTER INJECTION	PERCENTAGE EFFECT	2ND COUNT AFTER INJECTION	PERCENTAGE EFFECT
	drops	*drops*		*drops*	
6	8	10	+25.00	6	−25.00
12	24	13	−45.83	17	−29.16
17	20	11	−45.00	9	−55.00
28	3	10	+233.33	6	+100.00
33	6	5	−16.66	3	−50.00
37	17	15	−11.76	16	− 5.88
40	9	12	+33.33	8	−11.11
51	16	22	+37.50	No count	
58	8	4	−50.00	No count	

in all. The average decrease was 45.45 per cent. A further count was made in seven of these experiments and showed the effect to be maintained in all but one. The average decrease for this period below the initial count was 44.28 per cent.

In the seven cases in which secretin (table 6) was administered intravenously there was a marked augmentation in the amount of bile secreted. This increase averaged 241.52 per cent. A subsequent count was made on five occasions and in four of these the output of bile was still above the original level. Experiment 15 of this group is particularly striking; during the second period after the injection the amount of bile formed was six times as great as that during the first period and eighteen times that produced in the twenty minutes immediately preceding the giving of the secretin. The effect of secretin on this group of dogs is further confirmation of the results reported by Bayliss and Starling (2).

Splenic substance (table 7) was administered to seven animals and failed to exhibit any constant effect.

Thymus gland substance (table 8) uniformly brought about a decrease in the quantity of the biliary secretion during the first period after it was injected. This effect was still evident in three out of four experiments in which a second count after injection was made.

Intravenous injection of the substance of the thyroid gland (table 9), like splenic substance, was without constant effect.

A review of the literature has failed to reveal the report of any definite experiments with regard to the influence of the internal secretions on the amount of bile secreted. In a study of bile pigment metabolism Hooper and Whipple (3) state that in two groups of dogs, the members of one group having undergone splenectomy and those of the other group serving as controls, the quantity of bile pigment and of bile produced was identical. This result is in accord with our finding that splenic substance intravenously did not alter definitely the output of bile.

CONCLUSIONS

1. The amount of bile secreted is increased by secretin.
2. The amount of bile secreted is decreased by adrenalin and by mammary, orchic, ovarian, pancreatic and thymic gland substances.
3. The amount of bile secreted is not affected in a constant or definite manner by the substance of the spleen and thyroid gland.

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BIBLIOGRAPHY

(1) Downs and Eddy: This Journal, 1917, xliii, 416.
(2) Bayliss and Starling: Journ. Physiol., 1902, xxviii, 325.
(3) Hooper and Whipple: This Journal, 1917, xliii, 280.

BAKERS' YEAST AS FOOD FOR MAN[1]

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That brewers' yeast possesses food properties wsa demonstrated long ago. However it was not until the stress of war seriously reduced the available food supply of the world that the attention of scientists was directed to the yeast plant as a possible source of food protein. The Germans were the first to make a comprehensive study of its nutritive possibilities. Such investigators as Rubner (1), Schottelius (2), Wintz (3), Winckel (4), Fuchs (5), Kellner (6) and Völtz (7) testify to the dietetic importance of brewers' yeast, whereas Schrumpf (8) and others do not think it possesses much value as a food. Fendler and Borinski (9) are inclined to lay the difference in opinion of the above experimenters to the fact that the brewers' yeast is so variable in composition as to render impossible the comparison of data obtained from the use of yeast from different sources.

That the protein of yeast constitutes a suitable food for man is borne out by the investigation of its hydrolysis products (10). Such important amino acids as the following have been shown to be present: glycocoll, alanine, valine, leucine, phenylalanine, tyrosine, proline, aspartic acid, glutamic acid, tryptophan, lysine, arginine and histidine.

The yeast used by us in our studies possessed the following composition calculated on a water-free basis.

	per cent
Protein	52.41
Fat	1.72
Carbohydrate	37.13
Ash	8.74

The composition of the ash was as follows:

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[1] The expense of this investigation was defrayed in part by a grant from Mrs. M. H. Henderson.

Phosphorus (P_2O_5)	54.5
Silicon (SiO_2)	1.2
Calcium (CaO)	1.4
Magnesium (MgO)	5.2
Sulphur (SO_3)	0.5
Potassium (K_2O)	36.5
Sodium (Na_2O)	0.7
Chlorine	trace
Iron	trace

It is apparent therefore that this dried yeast has at least three times the calorific value of beef of moderate fat content (11). That dried yeast is readily digested and utilized by the animal organism has been shown by Rubner (12), Fuchs (13), Kellner (14) and others. According to Völtz (15) yeast is one of the most digestible of feeding stuffs and possesses excellent dietetic properties.

So far as metabolism experiments are concerned very few are on record. The most comprehensive of these was carried out by Funk, Lyle and McCaskey (16). The most important remaining ones were reported by Prausnitz and Menicanti (17) and Thomas (18). Funk and associates experimented upon four men who were fed a diet in which yeast[2] was the *sole source* of protein. They report that "a large part of the yeast nitrogen apparently has no food value" and that "it is badly assimilated" but conclude with the statement, "in the field of nutrition research our studies have by no means enabled us to pronounce a verdict that yeast possesses no value in dietetics."

In our own experiments which we report on the following pages, we studied the metabolism of six men and found that four of the six showed an improved nitrogen balance when fed the yeast diet. Moreover when all of our data on the six subjects were reviewed we found that the yeast diet had produced an average daily gain of about 0.4 gram of nitrogen per man, above that noted before yeast was fed. The only interpretation is that these men found yeast a satisfactory article of diet. It seems to us that there are several factors which serve to explain the diametrically opposed conclusions of Funk and ourselves. In the first place in our tests we fed the same amount of nitrogen per day throughout the normal periods and yeast periods, whereas the amount of nitrogen fed by Funk during the normal periods was always much higher than that fed during the yeast period. In the second place, Funk used yeast as the *sole source of protein,* whereas we substituted a certain amount

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[2] Obtained from American Pure Yeast Company of New York.

(9 per cent to 29 per cent) of the nitrogen of the normal diet by yeast nitrogen. In the third place, we used three types of diet while only one type was used in the other investigation. In the fourth place, Funk states that "the bad utilization of yeast may be partially due to the fact that the yeast had a disagreeable taste and was unpleasant to eat for any length of time." We avoided this feature by incorporating our yeast in wheat biscuits and in this way produced an yeast-wheat combination which had a most agreeable flavor. In fact the biscuits tasted better with the yeast than without it. And in the fifth place the kind of yeast used in the two studies was not the same, since Funk used a product furnished by the American Pure Yeast Company whereas we employed the ordinary bakers' yeast of commerce (Fleischmann). We feel that the divergence in the reports of Funk and ourselves was brought about by the influence of one or more of the above five factors.

TABLE 1

Approximate amounts of nitrogen ingested in the form of yeast (expressed as percentages of ingested nitrogen)

	EXPERIMENT					
	1		2		3	
Subject	F.	Sm.	H.	Sr.	G.	M.
Per cent	27	29	12	9	18	22

Preliminary baking tests. Before starting the experiments planned to study the influence of yeast upon the nutrition of man, it was necessary to make some preliminary baking tests in order to determine the influence upon the taste and physical characteristics of the loaf brought about by adding dried yeast to the flour used in bread making. These tests demonstrated that we could replace as high as 20 per cent of the flour by dry yeast and produce a loaf excellent in every way and possessed of an attractive flavor. This is equivalent to the replacement of 50 per cent of the protein of the flour.

In the various tests on men, from 9 per cent to 29 per cent of the nitrogen of the daily diet was replaced by this dried yeast (see table 1). The yeast was dried at 105°C. in a current of air and then put through proper milling processes to produce an yeast flour of the approximate fineness of ordinary wheat flour. The yeast flour mixed readily with the wheat flour, making a good homogeneous preparation. Excellent biscuits were prepared from this wheat-yeast flour.

We also made some tests upon the addition of yeast to meat preparations. In this connection we found that we could replace 25 per cent of the meat protein by yeast protein and have a nutritious and palatable mixture.

Experimental plan. The investigation was divided into three separate experiments, in each of which a study was made of the metabolism of two men. Each of these three experiments was again subdivided into from two to four individual experimental periods. In other words, a total of six different subjects was used in the three experiments and a total of eighteen periods was studied, the periods with two exceptions being seven days in length. All of the subjects were good reliable men who were thoroughly interested in both the scientific and practical aspects of the investigation.

In the first two periods of the *first experiment,* the men were fed a *high protein defective diet.* That is, they were given plenty of protein in the form of meat, wheat, etc. but the diet was not a *complete diet* from a strict nutrition viewpoint because it was lacking in the *indispensable water-soluble vitamine,* which is present in yeast and certain other foods. In the third period, the diet was supplemented by a small amount of yeast, (0.2 gram of yeast nitrogen) in order to add the essential vitamine just mentioned, and in the fourth period, a considerable part (27 per cent to 29 per cent) of the protein of the diet was replaced by yeast protein. In this period each subject received 8.43 grams of yeast nitrogen per day.

By the above procedure, the third period enabled us to study the value of the yeast vitamine when added in small amount to a defective diet, whereas period four enabled us to compare the digestibility or so-called "utilization" of yeast protein with the digestibility or "utilization" of proteins such as those of meat and wheat, which are customarily included in the diet of man.

The diet as fed consisted of meat, wheat biscuits, jelly, margarine, potato chips, crisco, corn starch, agar agar and water.

The actual cooking of the food was carried out by the head dietitian of Jefferson Hospital,[3] and accurately weighed amounts were fed to each man. Samples of all foods served were taken for chemical analysis and the analyses were always made in duplicate and when it was deemed necessary triplicate determinations were carried out. The urine was analyzed in twenty-four-hour periods. The feces were collected in thymolized receptables and frozen. Samples representing the individual periods were analyzed.

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[3] We are under obligation to Miss Gladwin for her aid in this connection.

The *second experiment* was planned to study the effect of yeast when added to a *low protein defective diet.* The diet was defective in that the "water-soluble vitamine" present in yeast and certain other foods was not included in sufficient amount.

The periods of "small" and "large" yeast addition were similar to those of the first yeast experiment. Here, however, only from 9 per cent to 12 per cent of the ingested nitrogen was in the form of yeast nitrogen. The urine and feces were collected and analyzed as already explained.

In the *third experiment,* the subjects were fed a normal complete diet, i.e., a diet which contained satisfactory protein, fat, carbohydrates, energy, inorganic salts and vitamines. The water-soluble vitamine was furnished by the use of *milk,* a food not included in the diet of the subjects of the first and second experiments.

In the period of large yeast ingestion in this test from 18 per cent to 22 per cent of the protein of the diet was replaced by the yeast protein.

For data regarding the nitrogen content of food, feces and urine of the three experiments, see table 2.

Discussion of the first experiment. In this experiment subjects "F" and "Sm" were employed. It being our desire to study the effect of yeast when added to and substituted in a high protein defective diet, we started the men on a diet containing over 16 grams of nitrogen per day (see table 2). This diet was so far in excess of the nutritional needs of the men that there was a daily gain of over 3 grams of nitrogen to the body. For this reason we ran an extra period in which the diet was cut to about 14 grams of nitrogen per day. The ingestion of this diet showed a daily gain of only 1.04 gram for "F" and one of 1.96 gram for "Sm."

With the above nitrogen balances as a basis we added a small amount of yeast to the diet. Each subject received only 0.24 gram of yeast nitrogen in a total daily nitrogen intake of about 14 grams. On this diet "F" gained 1.79 gram of nitrogen per day whereas "Sm" showed a daily gain of 1.67 gram.

In the final period of the experiment a considerable part of the protein of the diet was made up of yeast protein. The specially baked yeast biscuits, already mentioned, were used and from 27 per cent to 29 per cent of the ingested nitrogen was in the form of the nitrogen of yeast.

TABLE 2

Nitrogen balances

EXPERIMENT NUMBER	SUBJECT	NO YEAST PERIOD						NO YEAST PERIOD						SMALL AMOUNT OF YEAST PERIOD						LARGE AMOUNT OF YEAST PERIOD					
		Number of days	Nitrogen					Number of days	Nitrogen					Number of days	Nitrogen					Number of days	Nitrogen				
			Income	Outgo		Balance			Income	Outgo		Balance			Income	Outgo		Balance			Income	Outgo		Balance	
				Urine	Feces	Period	Daily			Urine	Feces	Period	Daily			Urine	Feces	Period	Daily			Urine	Feces	Period	Daily
			grams	grams	grams	grams	grams		grams	grams	grams	grams	grams		grams	grams	grams	grams	grams		grams	grams	grams	grams	grams
1				81.96	9.31					58.70	8.80					81.31	9.61					74.39	14.58		
	F.	7	114.2	91.3		+21.9	+3.13	5	72.7	67.5		+5.2	+1.04	7	103.4	90.9		+12.5	+1.79	7	106.1	89.0		+17.1	+2.44
				77.89	9.81					50.77	7.59					76.00	9.45					71.36	17.71		
	Sm.	7	114.2	87.7		+26.5	+2.78	5	68.7	58.4		+9.8	+1.96	7	97.2	85.4		+11.7	+1.67	7	99.8	89.1		+10.7	+1.35
2				60.31	6.78					46.75	7.48					46.49	7.18					46.14	8.92		
	H.	7	74.6	67.1		+7.5	+1.07	7	58.7	54.2		+4.5	+0.64	7	59.8	53.7		+6.1	+0.87	7	59.2	55.1		+4.1	+0.58
				68.47	10.68											69.11	11.27					62.95	10.06		
	Sr.	7	74.5	79.2		−4.7	−0.67							7	75.9	80.4		−4.5	−0.64	7	75.3	73.0		+2.3	+0.33
3				74.66	10.97																	71.10	14.81		
	G.	7	83.0	85.6		−2.6	−0.37													7	84.6	85.9		−1.3	−0.19
				49.54	9.90																	55.62	13.67		
	M.	6	60.	59.4		+0.8	+0.13													7	68.3	69.3		−1.0	−0.14

In the case of "F" there was a gain of 2.44 grams of nitrogen per day, as compared with a gain of only 1.04 gram during the period when no yeast was included in the diet (see table 2). In other words, when the nitrogen of the wheat and meat of the diet was substituted to the extent of 27 per cent by the nitrogen of yeast, the body was better nourished as shown by the gain of 2.44 grams of nitrogen per day. This more desirable condition was brought about in the case of "F" in spite of the fact that the yeast was given in such large amount as to produce a mild diarrhea. The stools were very soft and the daily output of fecal matter was increased to 992.1 grams from a value of only 637.6 grams for the period during which no yeast was eaten. This experiment thus furnished a very nice illustration of the laxative action of bakers' yeast, an action which we had previously demonstrated upon invalids (19).

Subject "Sm" showed a more pronounced diarrhea than did "F" and his nitrogen balance showed a daily gain of 1.53 gram. There is no question but what both of these subjects would have shown higher gains of nitrogen had not the laxative action of the yeast produced an excessive output of soft feces. That the utilization values and the figures in the nitrogen balance sheet do not necessarily run parallel is brought out nicely in the experiment on "F," as shown in table 3. In the first period when no yeast was included in the diet 87.9 per cent of the nitrogen of his food was absorbed from the bowel and 1.04 gram retained by the body. When a small amount of yeast was added to the diet he utilized more of the food nitrogen (90.7 per cent) and also retained more (1.79 gram). But the most remarkable demonstration came in the period in which a large amount of yeast was eaten. Here only 86.2 per cent of the food nitrogen was absorbed from the bowel *but the significant fact is that 2.44 grams of it was retained.*

The food eaten by "F" during the second "no yeast" period contained 72.7 grams of nitrogen (table 2). Of this amount 8.8 grams were lost in the feces, leaving 63.9 grams as absorbed. Of this 63.9 grams only 5.2 grams were retained in the body. In contrast to this condition we find that 17.1 grams out of total absorption of 91.52 grams were retained during the "large yeast" period. By calculating on the basis of the available data just cited, it will be found that 8.1 per cent of the nitrogen which was absorbed from the intestine in the "no yeast" period was retained in the body of "F" whereas 18.6 per cent of the absorbed nitrogen was retained when this absorbed nitrogen was more largely made up of the nitrogen of yeast. It seems, therefore, when the diet contained considerable yeast nitrogen that the body made better use of the food nitrogen than when no yeast was present. It is apparent that the yeast nitrogen was more satisfactory for the uses of "F" than was the nitrogen in the form of meat, wheat and the other foods used. The low utilization value (86.2 per cent) for "F" during the period of high yeast ingestion was due, as before stated, to the fact that the yeast had a pronounced laxative effect.

Subject "Sm" utilized 88.8 per cent of the nitrogen of his diet when no yeast was present whereas the utilization value rose to 90.3 per cent when a little yeast was added (table 3). In the high yeast period which followed there was a low utilization value (82.3 per cent) due to the laxative action of the yeast as already noted. For example, 872.5 grams of feces were passed during this period as against 496.4 grams when no yeast was present in the diet. Of course the bulk of this excess weight was water, as the stools were very soft. However, sufficient nutritive material was carried out of the bowel in the fluid mass to lower the utilization value appreciably.

TABLE 3

Summary of nitrogen balances and utilizations

EXPERIMENT NUMBER	SUBJECT		NO YEAST	SMALL AMOUNT OF YEAST	LARGE AMOUNT OF YEAST
1	F.	Daily balance (grams)	+1.04	+1.79	+2.44
		Utilization (per cent)	87.9	90.7	86.2
	Sm.	Daily balance (grams)	+1.96	+1.67	+1.53
		Utilization (per cent)	88.8	90.3	82.3
2	H.	Daily balance (grams)	+0.64	+0.87	+0.58
		Utilization (per cent)	87.2	87.9	84.9
	Sr.	Daily balance (grams)	−0.67	−0.64	+0.33
		Utilization (per cent)	85.7	85.3	86.7
3	G.	Daily balance (grams)	−0.37		−0.19
		Utilization (per cent)	86.8		82.5
	M.	Daily balance (grams)	+0.13		−0.14
		Utilization (per cent)	83.5		80.0

It is quite significant that in spite of the large loss of water from the body in the soft feces, "F" *gained three pounds in body weight* on the yeast diet and "Sm" gained one pound. Schottelius (20) reports gains in body weight by five out of nine subjects after yeast ingestion.

Discussion of the second experiment. In this experiment we studied the influence of yeast when added to and substituted in a low protein defective diet. The men used in these tests were "H" and "Sr." We started "H" out upon a diet containing only a little over 10 grams of nitrogen per day (see table 2) and notwithstanding this very low food intake his body retained 1.07 gram of nitrogen per day. We then ran an extra period in which the nitrogen content of the diet of "H" was cut to a little over 8 grams per day.

On this very low diet "H" gained 0.64 gram of nitrogen per day whereas "Sr" lost 0.67 gram of nitrogen per day on a diet containing 10.64 grams of nitrogen.

When a small amount of yeast was added to the diet the nitrogen balance sheets (tables 2 and 3) show that "H" retained more nitrogen (0.87 gram) than when no yeast was present. The nitrogen retention of the high yeast period was about the same (0.58 gram) as that for the period in which no yeast was eaten.

Subject "Sr" was losing nitrogen (0.67 gram daily) at the time the small amount of yeast was added to his food. There was a smaller loss of nitrogen per day (0.64 gram) after the yeast was added, but the difference was not significant. However, when the diet was so changed that yeast nitrogen was substituted for 9 per cent of the nitrogen of the meat and wheat of the diet, the balance very quickly shifted from a negative to a positive one. In other words, the data show that the body *lost* 0.67 gram of nitrogen every day for a week when no yeast was present in the diet whereas there was a *daily gain* of 0.33 gram of nitrogen for the period in which the large amount of yeast was eaten.

In the case of "Sr" the utilization values run parallel with the nitrogen balances. In the "no yeast" and "small yeast" periods a little over 85 per cent of the nitrogen of the diet was absorbed from the bowel while 86.7 per cent of the food nitrogen was absorbed when 9 per cent of yeast nitrogen was present. We thus see that when the diet had a high yeast value that the body of "Sr" showed better absorption from the bowel and likewise a higher retention of nitrogen by the cells of the body. It is interesting to note that "Sr" was one of the "furunculosis" patients in our therapeutic tests (21). In the case of "boils" the yeast produced a rapid and complete cure.

Although "H" was ingesting less food than "Sr," yet the percentage of the food nitrogen which was in the form of yeast nitrogen was 12 per cent in the high yeast period as against 9 per cent for "Sr." The utilization figure for this period was 84.9 per cent as against 87.2 per cent for the "no yeast" period and 87.9 per cent for the period of "small yeast" intake. The nitrogen balance for the "high yeast" period was about the same as that for the "no yeast" period.

The yeast ingestion was not sufficiently high in this experiment to produce the laxative effect noted in the first experiment.

Discussion of the third experiment. In this experiment we studied the influence of yeast when substituted for protein from other sources in a diet entirely complete and satisfactory in every way. Only two periods were run inasmuch as the period of "small yeast" was not necessary as the water-soluble vitamine was already present in the milk of the diet. The subjects of the experiments were "G" and "M."

Subject "G" was fed a diet containing about 12 grams of nitrogen (see table 2). On this diet he showed a negative nitrogen balance of 0.37 gram per day when no yeast was present in the diet. In the next period 18 per cent of the nitrogen in the form of wheat and meat was replaced by yeast nitrogen. A week on this diet yielded a minus balance of 0.19 gram per day. Apparently the yeast was as satisfactory for the nutritional uses of this man as was beef and wheat, notwithstanding the fact that the utilization value of the yeast period (82.5 per cent) was lower than that of the "no yeast" interval.

The diet of "M" contained about 10 grams of nitrogen (see table 2). On this diet he gained 0.13 gram of nitrogen per day and lost a similar quantity (0.14 gram) when 22 per cent of the nitrogen of this diet was in the form of yeast. There was also a somewhat lower utilization value (80.0 per cent) than when no yeast was eaten (83.5 per cent). Inasmuch as the diet of subjects "G" and "M" was perfectly complete from the nutritional standpoint without the addition of yeast it is not surprising that the yeast did not cause an increased nitrogen retention. No laxative action was observed in the case of the subjects of this experiment.

CONCLUSIONS

Bakers' compressed yeast forms a very satisfactory article of diet for man.

The yeast may be incorporated with meats such as hamburg steak and yields a preparation of very satisfactory taste.

If the yeast be dried in a current of air at 105°C. and pulverized it may be substituted for 20 per cent of the wheat flour used in bread making and yields a product which is very nutritious and of an attractive flavor.

From 10 to 30 per cent of the nitrogen of an ordinary mixed diet may be replaced by yeast nitrogen in the form of compressed yeast without detriment to the best nutritive interests of the individual.

We have demonstrated that the nitrogen of yeast is preferred by the nutritional mechanism of certain individuals, to the nitrogen in the form of such staple foods as meat and wheat. In the case of certain other individuals no differentiation is possible between the nutritive value of yeast and the staple foods mentioned.

The average individual may ingest yeast sufficient to yield from 1 to 2 grams of nitrogen per day, without securing any laxative effect. When as high as 4 grams of yeast nitrogen are eaten, a laxative effect is generally observed and in some cases soft diarrheal stools result. This laxative action of yeast may be put to good use in overcoming constipation.

When we take into consideration the experiments on all six of the men who served as subjects, we find that four of the six found the diet more satisfactory when yeast was included. In other words, subjects "F," "H," "Sr" and "G" showed an improved balance when yeast was present in the diet, whereas the differences in the balances of "M" are not significant and the variation in the balances of "Sm" is due to the fact that the subject was fed such a large amount (29 per cent) of yeast as to cause the passage of watery stools.

When all of the experimental periods are reviewed we find that the average daily gain of nitrogen when no yeast was present in the diet was 0.48 gram, whereas this gain was increased to 0.82 gram when yeast was eaten along with other foods.

Of the six subjects who ate the yeast diet, four made gains of from ½ pound to 3 pounds in body weight, in a period of two weeks, while the other two men showed a loss.

The relation of yeast to the growth of the animal body will be discussed in a subsequent paper.

The authors are grateful to Dr. Olaf Bergeim and to the subjects of the experiment for assistance given them.

BIBLIOGRAPHY

(1) Rubner: Münch. med. Wochenschr., 1916, lxiii, 629.
(2) Schottelius: Deutsch. med. Wochenschr., 1915, xli, 817.
(3) Wintz: Münch. med. Wochenschr., 1916, lxiii, 445.
(4) Winckel: Die Wirtschaftliche Bedeutung der Hefe als Nahrungs-, Futter- und Heilmittel, München, 1916.

(5) Fuchs: Cited by Winckel.
(6) Kellner: Cited by Winckel.
(7) Völtz: Zeitschr. Spiritusind., 1916, xxxix, 53, 64.
(8) Schrumpf: Münch. med. Wochenschr., 1916, lxiii, 296.
(9) Fendler und Borinski: Deutsch. med. Wochenschr., 1916, xlii, 670.
(10) Pringsheim: Woch. Brauerei, 1913, xxx, 399.
Meisenheimer: Woch. Brauerei, 1915, xxxii, 325.
Neuberg: Woch. Brauerei, 1915, xxxii, 317.
Winckel: Loc. cit.
(11) Schottelius: Loc. cit.
(12) Rubner: Loc. cit.
(13) Fuchs: Loc. cit.
(14) Kellner: Loc. cit.
(15) Völtz: Loc. cit.
(16) Funk, Lyle and McCaskey: Journ. Biol. Chem., 1916, xxvii, 173.
(17) Prausnitz and Menicanti: Zeitschr. Biol., 1894. xxx, 340.
(18) Thomas: Arch. Anat. u. Physiol., 1909, 219.
(19) Hawk, Knowles, Rehfuss and Clarke: Journ. Amer. Med. Assoc., 1917, lxix, 1243.
(20) Schottelius: Loc. cit.
(21) Hawk, Knowles, Rehfuss and Clarke: Loc. cit.

COMPRESSED YEAST AS FOOD FOR THE GROWING ORGANISM[1]

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Various experiments (1) made in this country and abroad have demonstrated beyond question the importance of so-called accessory food substances or vitamines in the growth-producing dietary. One of the earliest of these to be demonstrated was the vitamines of yeast (2). Later Funk, Funk and Macallum and Osborne and Mendel directed our attention anew to this important property of yeast. In each instance the above workers used brewers' yeast.

In our own experiments, which we herewith report, the ordinary household compressed yeast (Fleischmann) was utilized and was demonstrated to have very important growth-promoting properties.

DESCRIPTION

Experimental animals. Thirty-two white rats, ranging from 30 to 40 days of age were purchased from the white rat colony of the Wistar Institute. The thirty-two rats were included in six litters and were evenly divided as to sex, sixteen being males and a like number females.

For experimental purposes we divided the rats into four groups. Males and females were kept separate. Our experiments therefore entailed the use of two groups of males and two groups of females. So far as possible care was taken to include at least one rat from each litter in each of our four experimental groups. Each rat was given a number and was further marked for easy identification by the usual method of slitting the ear. The body weight of each animal was accurately determined upon a torsion balance at the start of the experiment and at short intervals thereafter.

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[1] The expenses of this investigation were defrayed in part by a grant from Mrs. M. H. Henderson.

Preparation of diet. Four diets as shown in table 1 were fed. These were "meat diet," "casein diet," "meat and yeast diet," and "casein and yeast diet."

The *meat* used was good "round steak." This was bought in large quantity, the muscle separated and all fat and connective tissue stripped carefully from the lean portion. Much time and care were used in following out the small connective tissue septi, so that the final product was only the lean muscle part. This was twice ground very fine in an electric meat grinder and the resultant mass mixed by hand for one hour to insure a homogeneous product. This ground meat was then dried by spreading it in a thin layer over a broad platter placed over an electric plate set at low heat. More rapid drying was secured by allowing the air current from an electric fan to play upon the thinly spread meat layer. When dry enough to break up readily the meat was placed in a small ventilated electric oven and heated over night at 100° to 105°C. It was then ground to a fine powder and again mixed thoroughly. This dried meat powder was then used in making up the "meat diet," and the "meat and yeast diet."

TABLE 1

List of diets employed (values in percentages)

CONSTITUENTS	DIETS			
	Casein	Casein-Yeast	Meat	Meat-Yeast
Meat			20	20
Casein	20	20		
Starch	63	63	63	63
Butter fat	15	15	15	15
Salt mixture	2	2	2	2
Yeast (dry)		5 per cent added		5 per cent added
Nitrogen content of diet	2.79	3.17	2.70	3.12

In preparing the *casein* for use in the experiments, commercial casein was soaked in a large volume of water for twelve hours and then dissolved by stirring in dilute sodium hydroxide. The casein was precipitated by adding dilute acetic acid very carefully and with constant stirring until the maximum precipitate was obtained. After settling, the supernatant liquid was siphoned off. This process of solution and precipitation was repeated three times. The final casein precipitate was washed again with water, followed by alcohol and ether in turn. The casein was then dried, ground and mixed as already described for meat, and the resultant casein powder was used in making up the "casein diet," and "casein and yeast diet."

The *butter fat* used in the diets was obtained by separating the melted fat of *unsalted* butter from the sediment of curd and water. The *salt mixture* (3) was prepared from chemically pure salts and had the composition shown in table 2.

The dry materials for the diet were mixed very thoroughly in a large mixing bowl, and melted butter was added slowly with constant stirring. The food mixture was then sieved to break up any lumps that formed on the addition of the butter-fat. The mixed foods, as well as the individual dietary constituents such as meat and casein powders, were kept in air-tight containers in a freezing tank.

TABLE 2

Salt mixture

SALT	AMOUNT
	grams
Calcium carbonate	134.8
Magnesium carbonate	24.2
Sodium carbonate	34.2
Potassium carbonate	141.3
Phosphoric acid	103.2
Hydrochloric acid	53.4
Sulphuric acid	9.2
Citric acid	111.1
Ferric citrate	6.34
Potassium iodide	0.020
Manganese sulphate	0.079
Sodium fluoride	0.248
Potassium aluminium sulphate	0.0245

The yeast added to the diet was a per cent quantity by weight of the total diet. For example, 5 per cent of dried yeast by weight was incorporated into the mixture of dry substances (casein or meat, starch and salt-mixture) before the addition of the melted butter.

The diets as fed and their nitrogen contents are given in table 1.

Experiments on group I. This group included nine male rats, no. 1 to 9 inclusive. It was planned to feed these rats for a time on a diet which was inadequate for growth and when the failure to grow at the normal rate had been established to add a little yeast to the diet and see if normal growth followed. The diet fed was the "casein diet" listed in table 2. This diet included 20 per cent casein, 63 per cent starch, 15 per cent butter and 2 per cent salt mixture (see table 1). This diet is not satisfactory for growth because it lacks "water-soluble B."

The diet as we fed it was really an artificial milk so far as food value was concerned with the exception that the "water-soluble B" of the milk was not present.

TABLE 3

Experiments on group I. Males, cage 1, casein

RAT NUMBER	1	2	3	4	5	6	7	8	9
LITTER NUMBER	1	1	2	3	3	3	5	6	6
DATE	WEIGHT								
1918	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
February 21	40	41	58	31	28	29	59	40	35
February 28	43	47	70	31	30	32	73	46	45
March 7	42	47	75	29	30	30	70	*	48
Casein and yeast, March 7, 1918									
March 11	43	53	79	31	35	32	76		52
March 14	45	56	90	37	37	32†	72		53
March 18	46	57	98	41	40	‡	82		52
March 21	49	63	110	45	43		91		52
March 28	49	67	118	47	53		102		§
April 4	70	92	149	68	67		134		
April 11	93	111	170	87	91		159		
April 18	114	130	192	113	113		177		

* Dead, March 6, 1918.

† Sick.

‡ Dead, March 15, 1918.

§ Chloroformed March 28, 1918.

Table 3 contains the observations from the animals in group I. At this stage in the life cycle of the young rat, it should about double its weight in an interval of fourteen days. However, none of these rats in any way approached such gains in body weight. In fact, some of them showed an actual loss and the maximum gain was one from 58 grams to 75 grams shown by rat 5. This was a gain of less than 30 per cent whereas the normal gain should have been about 100 per cent. On the other hand, rat 4 showed an actual drop in body weight from 31 grams to 29 grams and rat 6 also dropped in body weight from 32 grams to 30 grams in seven days.

After two weeks a little yeast was added to the diet and every rat showed an immediate gain in weight as may be seen by examining table 3. The body weight gains were not very large at first because of the pronounced "nutritional shock" which the eating of the inadequate diet gave the young rats in their very early days. That is, they about doubled their weight in four weeks whereas other rats from the same litters when fed the same diet in their early days more than doubled in weight in two weeks (see table 4). The preliminary "stunting" of the rats by the defective diet wore off in large part at the end of the fourth week of the yeast diet and a rapid growth developed. Rat 3, for example, weighed 75 grams at the start of the yeast diet and six weeks later his body weight had mounted to 192 grams, whereas rat 5,

TABLE 4

Experiments on group II. Males, cage II, casein and yeast

RAT NUMBER	10	11	12	13	14	15	16
LITTER NUMBER	1	1	2	3	3	5	6
DATE	WEIGHT						
1918	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
February 21	39	38	57	30	31	57	52
February 28	64	59	83	52	58	89	79
March 7	88	81	120	78	85	114	103
Casein March 7							
March 11	83	75	121	76	86	111	103
March 14	82	74	123	75	85	105	102
March 18	73	63	122	70	83	98	95
March 21	71	63	121	67	80	93	91
March 28	61	54	114	59	72	84	79
Casein and yeast March 28							
April 4	80	68	145	80	96	112	105
April 11	98	81	167	98	125	137	124
April 18	125	114	190	128	157	168	151
April 25	147	128	205	149	162	192	165
May 2	153	142	214	165	172	205	177
May 9	168	152	223	173	181	210	185

which weighed only 30 grams at the start of the yeast diet, showed a body weight of 113 grams six weeks later. The growth curve of this rat is reproduced in chart 1.

In our experiments on group I nearly all the rats *maintained* their body weight during the first two weeks of the experiment. Had they been *adult* rats this condition would have been satisfactory. However, they were *very young* rats and needed a diet adequate for growth and such a diet was furnished as soon as we added a little yeast with its water-soluble vitamine.

Experiments on group II. This group included seven male rats, no. 10 to 16 inclusive. The animals were given an adequate diet of casein and yeast as listed in table 1. This diet was satisfactory for growth because of the presence of the yeast and every rat showed immediate and pronounced increases in body weight. In fact, every rat doubled in weight in two weeks and rat 14, in growing from 31 grams to 85 grams in two weeks, *nearly tripled its weight.*

At the end of the fourteen days the yeast was eliminated from the diet and the effect upon the nutrition of the animals was very marked. Not only did the very rapid growth cease but there was *an actual loss in weight.* Thus rat 14 which had gained 54 grams in two weeks while eating a diet containing yeast, showed a *loss of 5 grams* when he was no longer fed yeast. The loss in weight was very nicely shown in the case of rat 11. This rat increased in weight from 38 grams to 81 grams during the two weeks of yeast feeding and decreased to 54 grams at the end of three weeks' feeding on a yeastless diet. The growth curve of this animal is shown in chart 1.

In order to try the influence of yeast at a later stage in the life of the rats, a third period was started at this point and the original diet of casein and yeast was again fed. The same rapid growth was again in evidence throughout an interval of six weeks in spite of the fact that the rats had been "stunted" by eating an inadequate diet for a period of three weeks just previously. In fact, some of the rats showed a weight well above 200 grams at the end of the period.

Experiments on group III. Nine female rats, no. 17 to 25 inclusive, were employed in this group. They were fed the "meat diet" listed in table 1. This diet was the same as the "casein diet "with the exception that round steak, dried and powdered was substituted for the casein.

On this diet the rats failed to show normal growth (4) although their growth was rather better than that shown by rats from the same litters when fed the "casein diet." Rat 24 is a notable exception (see table 5). This rat weighed 53 grams at the time she was placed on the meat diet and weighed only 55 grams over a month later. Every rat showed initial body weight gains of varying degree but in all instances these initial gains were followed by ultimate losses at the end of about a month. In other words, a diet containing good beef, butter, mineral matter, plenty of energy and an abundance of the important vitamine "fat-soluble A," was not adequate for the growth of the rats.

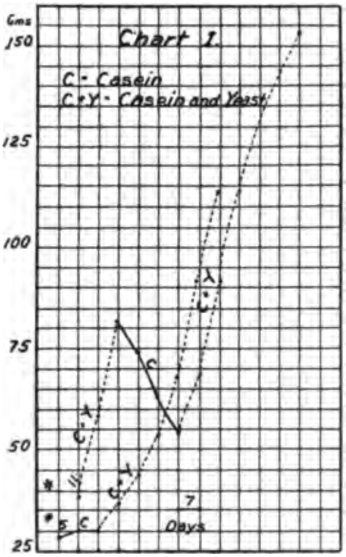

Chart 1

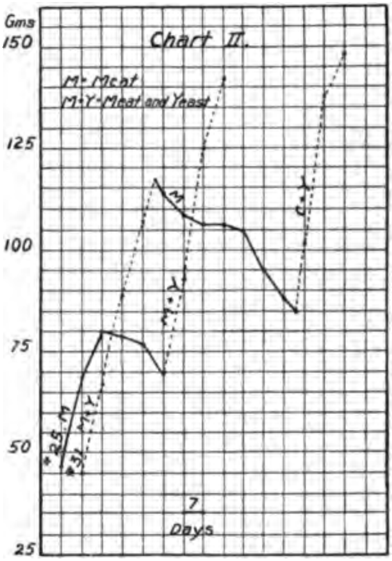

Chart 2

However, the addition of a little yeast made the diet adequate and the rats grew rapidly as shown by the tabulated data in table 5. *Rat 25, for example, lost 8 grams in weight during the last two weeks upon the meat diet and gained 55 grams in the two weeks immediately following simply through the addition of a little yeast. (See chart 2.)* Other gains almost as spectacular are recorded in table 5, all of which further emphasize the fact that yeast possesses unusual power to promote growth.

The case of rat 24 is worthy of special note. While on the diet of meat and yeast this rat contracted pneumonia. She was isolated weighing 105 grams and two weeks later in spite of her condition she weighed 130 grams.

Experiments on group IV. Seven female rats, no. 26 to 32, were used in these experiments. They were first fed a diet of meat and yeast (see table 1). On this diet they showed very satisfactory growth as will be seen by examining the data contained in table 6.

TABLE 5

Experiments on group III. Females, cage III, meat

RAT NUMBER	17	18	19	20	21	22	23	24	25
LITTER NUMBER	1	2	2	3	3	4	4	5	6
DATE	WEIGHT								
1918	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
February 21	38	54	51	31	31	36	42	53	46
February 28	60	77	74	47	45	54	62	76	68
March 7	72	86	85	51	45	69	72	65	80
March 14	75	83	89	49	35*	67	71	62	79
March 18	75	86	90	48	†	65	71	61	78
March 21	72	86	96	48		65	70	58	78
March 25	69	92	99	49		63	65	55	78
	Changed March 25, meat plus yeast					Changed March 25, meat plus yeast			
April 1	85	90	97	37*		84	74	65	70
		Changed April 1, meat plus yeast							Changed April 1, meat plus yeast
April 8	92	110	113			105	95	92	93
April 15	100	124	131			130	119	105*	125
April 22	102	127	132			130	127		142
April 29	105	134	138			132	132	130	147

* Sick.

† Dead March 15, 1918.

When the yeast was no longer fed, the rats lost weight as in the previous tests. For example, rat 31 grew from a weight of 45 grams to one of 117 grams between February 21 and March 18 when fed a yeast diet, whereas her weight decreased from 117 grams to 85 grams between

March 18 and May 6, when fed *a yeastless* diet. Chart 2 shows the growth curve of this rat.

TABLE 6

Experiments on group IV. Females, cage IV, meat plus yeast

RAT NUMBER	26	27	28	29	30	31	32
LITTER NUMBER	1	2	2	3	3	4	5
DATE	WEIGHT						
1918	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
February 21	36	55	54	32	29	45	51
February 28	57	82	82	54	51	66	82
March 7	40	108	100	72	67	89	100
March 14	*	122	109	65§	68	106	114
March 18	†	126	124		73	117	126
March 21				66‡			
Meat March 18							
March 21		128	126		75§	113	126
March 28		128	115		63§	108	103§
April 4		127	117		76§	106	
April 11		130	114			106	
April 18		129	109		94	105	
April 25		130	102		90	95	
May 2		134	102		89	88	
May 6		128	97		89	85	
Casein and yeast May 6							
May 9		146	113		98	101	
May 13		157	126		108	120	
May 16		159	137		117	137	
May 23		166	145		130	148	

* Dead March 8, 1918.

† Dysentery.

‡ Dead.

§ Sick.

A third period of seventeen days was used in this experiment. During this interval each rat was fed the usual casein-yeast diet and the customary gains in body weight were observed.

CONCLUSIONS

The addition of compressed yeast to a diet lacking the water-soluble vitamine causes an immediate and pronounced increase in body weight. This increase is more rapid and pronounced when the diet contains casein than when the protein of the diet is furnished by lean meat.

Compressed yeast may be heated to 105°C. without losing its growth-promoting properties.

The authors are under obligation to Dr. Clarence A. Smith for assistance rendered.

BIBLIOGRAPHY

(1) Hopkins: Journ. Physiol., 1912, xliv, 425.
Funk: Journ. Biol. Chem., 1916, xxvii, 1.
Funk and Macallum: Journ. Biol. Chem., 1915, xxiii, 413; 1916, xxvii, 51.
Osborne and Mendel: Journ. Biol. Chem., 1917, xxxi, 149; 1917, xxxii, 309.
(2) Hopkins: Loc. cit.
(3) Osborne and Mendel: Journ. Biol. Chem., 1917, xxxii, 309.
(4) Osborne and Mendel: Journ. Biol. Chem., 1917, xxxii, 309.

ALTERATIONS IN THE ACTIVITY OF THE TERRAPIN'S HEART RELATIVE TO SLIGHT CHANGES IN THE P_H VALUE OF THE PERFUSATE

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The influence upon the beating heart of a change in the reaction of the perfusate has been studied by a number of investigators. Thus Gaskell (1) reported in 1880 that perfusion with dilute alkali caused the heart to stop in systole, while the effect of a weak acid was to stop it in diastole. Mines (2) reviewed the effect of the hydrogen ion concentration and made a study of its action upon the various phases of the heart beat. Jerusalem and Starling (3), working upon the isolated heart of the frog, found that CO_2 in an excess of oxygen had an injurious effect, causing a relaxation which definitely diminished the ventricular output. These authors believed, however, that a certain tension of CO_2 in the blood was beneficial to activity. Ketcham, King and Hooker (4) found that CO_2, 2.5 per cent in an excess of oxygen, depressed the activity of the isolated terrapin's heart and that 3 per cent of the same had a similar effect upon the isolated mammalian heart. Mines (5) attempted to reconcile the apparently divergent results of Ketcham, King and Hooker, and Jerusalem and Starling upon the supposition that the latter had started with a perfusate which was too alkaline and the beneficial action reported by them he ascribed to the action of the CO_2 in slightly increasing the P_H[1] value to a level nearer the optimum. Mines showed that a heart perfused with a Ringer mixture of $P_H = 10.2$ was greatly benefited by the addition of sufficient CO_2 to raise the P_H to 6.9. However, if "neutral Ringer" were used to start with, any addition of CO_2 caused a depression of activity.

Clark (6) found that when CO_2 was bubbled through Ringer in a quantity sufficient to raise the P_H from 8.3 to 6.7, a markedly beneficial result was often obtained upon perfusion. This he laid to a specific action of the CO_2 upon the heart and not to any increase in the P_H of the solution.

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[1] It may be pointed out that P_H is simply the cologarithm, i.e., the logarithm of the reciprocal of the C_H. Thus C_H $0.35 \times 10^{-7} = P_H$ 7.45.

Two features of the work thus far published upon the influence of a change of the hydrogen ion concentration upon the heart are to be noted. In the first place those investigators who have expressed the characteristics of their perfusates in percentage CO_2 have not stated the corresponding P_H values. Clark points out that Ringer's mixture containing $NaHCO_3$ 0.01 per cent and NaH_2PO_4 0.001 per cent has a P_H value of 8.3, and that addition of 1 per cent CO_2 will raise the P_H to 7.0, while the further addition of 1 per cent raises it to 6.6. So that the workers who have not measured the actual change in the P_H value have probably dealt with acid solutions and the deleterious results observed were due to the acidity. In the second place, those who have measured the P_H value of their solutions have worked with differences in P_H value far greater than any which could take place in the blood even in cases of severe acidosis.

It has seemed desirable, therefore, to make a study of the effect of variations of the P_H of the perfusate within substantially normal limits. To this end a series of experiments was carried out on the heart of the terrapin.

EXPERIMENTAL

The heart was disclosed after removal of the plastron and a cannula inserted in a vena cava. A second cannula for outflow was inserted in the aorta. The perfusion pressure was regulated by Marriott bottles and manometer or by means of a chimney cannula after Mines. In all cases the pressure was kept as nearly constant as possible especially during the change from one perfusate to another and at a level just sufficient to cause dilatation of the auricles in diastole and yet permit contraction in systole.

Record was made of the beat of both auricles and the effect of tone changes in one chamber upon the record of another was as far as possible avoided. One side of the ventricle was anchored and the beat recorded by means of a thread from the other side. The auricular beats were recorded by means of a thread led off from the tip of each chamber. By means of pulleys the three chambers were made to record their beats simultaneously upon a smoked surface.

Ringer's solution was made up in quantities of six to twelve liters according to the following formula:

NaCl	0.7	$NaHCO_3$	0.02
KCl	0.25	NaH_2PO_4	0.001
$CaCl_2$	0.20	Water to	100

Such a mixture was found to have a P_H from 7.8 to 8.0. The desired P_H was then obtained by removing a portion to a smaller bottle and adding M/10 HCl or M/5 $NaHCO_3$. The P_H was measured by adding five drops of a 0.01 per cent solution of phenolsulphonephthalein to 3 cc. of the Ringer's and comparing with standard tubes. Within the range used in these experiments a change of P_H of 0.1 could be easily detected.

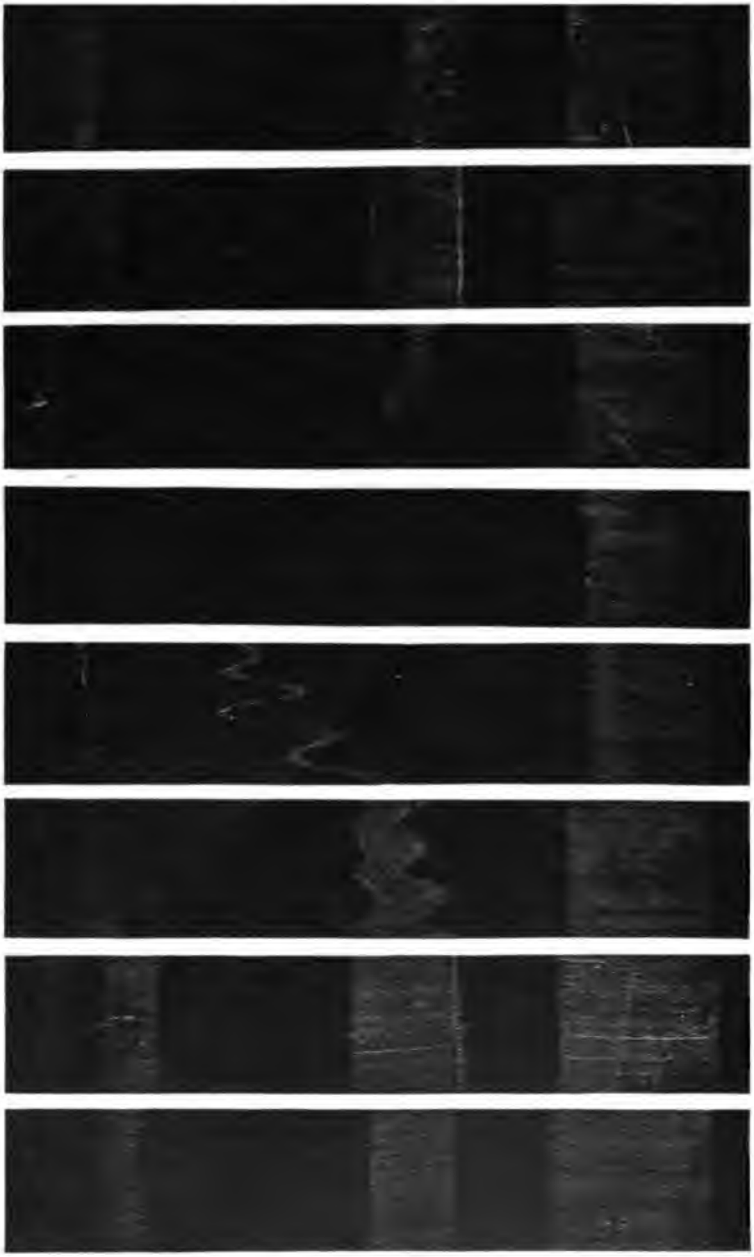
The P_H of the blood of the terrapin used, determined by the method of Levy, Rowntree and Marriott (7), was found to be 7.4 to 7.5. This was taken as the normal for the perfusates and variations were made to both sides. In no case, however, was a P_H of less than 8.0 or more than 7.1 employed. The majority of the experiments involved changes from 7.3 to 7.6.

The results of a series of experiments are shown in the accompanying charts and figures.

The record in figure 1 shows, in general, the results of such changes in the P_H of the perfusate. The upper record registers the beat of the left auricle, the middle that of the right and the lower that of the ventricle. A solution of P_H 7.4—approximately that of the blood—was passed through the heart for a half-hour and was then replaced by one of P_H 7.3. There followed at once an increase in the amplitude of the beat in all chambers. This was most striking in the right auricle where the height of the record of beat increased from 13 to 18 mm. in ten minutes. In the ventricle the increase was not so great but was easily perceptible.

At the arrow a perfusate of P_H 7.5 was introduced with very characteristic results,—a falling off in the amplitude of beat of all chambers and a gradual increase in tonus. Further decrease in P_H, to 7.7 and 7.8 as indicated upon the record, served to accentuate these results until in the ventricle the amplitude of contraction had been reduced by a half and there was a perceptible increase in tonus. The auricles soon decreased in size so that their beats could no longer be recorded.

When this condition had been reached the P_H was raised to 7.3. The tonus, both in the auricles and ventricle, was at once lowered and the amplitude slowly increased until, fifteen minutes later, the beats were again strong and regular. At the arrow Ringer of the same P_H as the blood was again introduced with a slight decrease in the amplitude in all chambers.



H 3:10 P_H 7.4

G 2:55 P_H 7.3

F 2:45 P_H 7.3

E 2:30 P_H 7.8

D 2:10 P_H 7.7

C 1:50 P_H 7.5

B 1:40 P_H 7.3

A 1:10 P_H 7.4

Fig. 1

In the experiment from which figure 2 was taken a series of relatively rapid changes of P_H was made. Perfusion commenced at a P_H of 7.4 and after ten minutes this was lowered to 7.6 for twenty minutes. This caused an increase in tonus and a decrease in amplitude as usual. Change to P_H 7.3 for a half-hour produced some relaxation but not sufficient to bring the tonus back to the normal level. Upon lowering to P_H 7.4 for ten minutes there was some rise in tonus. P_H 7.6 for ten minutes caused a sudden large rise which however fell off sharply when a perfusate of P_H 7.4 was introduced. After ten minutes of this a P_H of 7.3 caused some further relaxation. A change to P_H 7.5 caused a slow but very definite increase in tonus which was then decreased by a P_H of 7.4.

Here the increase in tonus in the auricles produced by the more alkaline perfusates was not entirely balanced by the action of those slightly more acid than the blood so that the tonus level at the end of the experiment was somewhat higher than at the start. As in the former experiments, the changes produced in the ventricle were smaller and somewhat more sluggish than those in the auricles.

These results point clearly to the action of slight alterations of P_H of the fluid bathing the heart upon two important phases of its activity, namely, tonus and amplitude. There is invariably a rise in tonus following a decrease in the H-ion content of the perfusate, while a slight increase in acidity causes a corresponding fall in the tonus level. There is an accompanying effect upon the amplitude of contraction though by no means as regular nor as marked as that upon the tonus. But in general there is an increase in amplitude with the more acid perfusates and a decrease with the more alkaline. This result may be due not entirely to the direct effect of the P_H upon the contractile tissue but partly to intra-auricular and intra-ventricular tension. Rate was not perceptibly affected.

It should be noted that in no case was the perfusate made actually acid—a P_H of less than 7.0—but merely somewhat more acid than the blood. The injurious effects of an acid perfusate as observed by Mines (2) were therefore not encountered.

Gaskell (8) has shown that when an alkaline perfusate is passed through the blood vessels there is a decrease in outflow due to an increase in the vascular tone. When an acid solution is used the opposite occurs. Since the heart has embryologically the same muscle layers as the blood vessels and is formed from a single vessel, it is not unreasonable to suppose that it might be similarly affected. And, indeed, this has been shown to be the case.

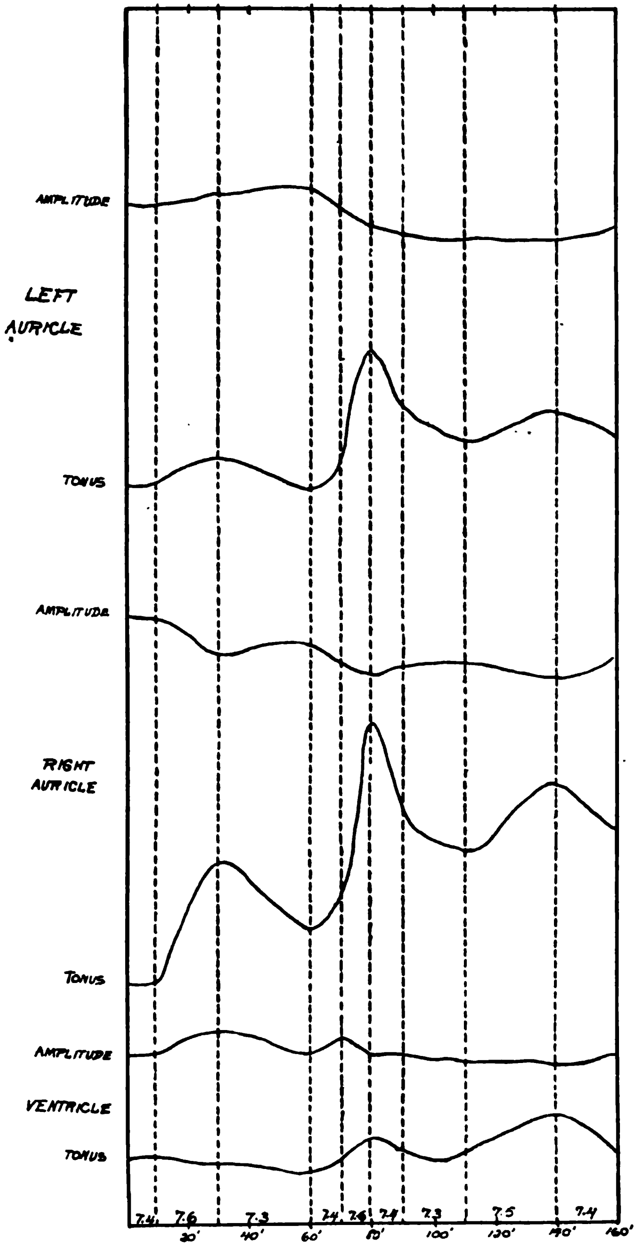

AMPLITUDE
LEFT AURICLE
TONUS
AMPLITUDE
RIGHT AURICLE
TONUS
AMPLITUDE
VENTRICLE
TONUS
7.4
7.6
7.3
7.4
7.6
7.4
7.3
7.5
7.4
20'
40'
60'
80'
100'
120'
140'
160'

Fig 2

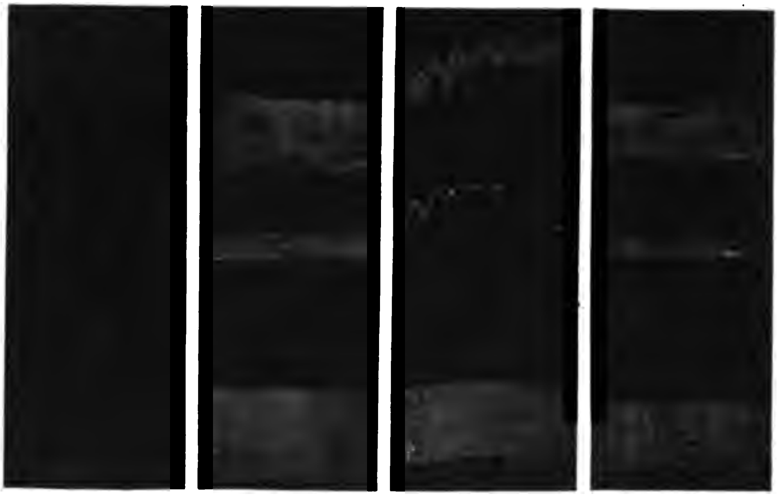
Gesell (9) has made a most careful study of the effect of tone changes upon the cardiac output and finds that perfusion of the terrapin's heart with a constant venous pressure shows variations in ventricular output resulting from changes in auricular tone. This he attributes to actual changes in the auricular output, which cause greater length and tension of the ventricular fibers, thereby producing greater efficiency of ventricular systole. He points out that as auricular tone is reduced the output per beat increases. To quote,

> The long muscle is more efficient than the short. The volume of the auricle increases as the cube, its surface as the square of the radius. Therefore, the greater the volume of the auricle the greater will be the volume change per unit length of shortening of auricular fiber.

Hence in the experiments above, regardless of any increase in the amplitude of contraction, the volume output of the auricles was increased with the more acid solutions. As Gesell has also pointed out, such an increase would produce a greater length and tension of ventricular fiber and hence a more efficient ventricular systole. In some instances this would amount to an actual increase in the amplitude of the ventricular contraction. The amplitude effects on the ventricle in some of the experiments mentioned above may be explained in this way.

But there was a very definite tonus effect in the ventricle entirely independent of the filling action of the auricular systole. In the experiment, the record of which is shown in figure 3, A and B, there was a condition of reversed sequence in which the ventricle led, the sequence being ventricle-right auricle-left auricle, and both auricular systoles occurred during the contraction phase of the ventricular beat (see fig. 3 A). The effect of the auricular output upon the length and tension of ventricular fiber was thus entirely eliminated. But substantially the same results as regards tonus were observed as in the cases where the hearts were beating in normal sequence, i.e., an increase in tone with greater alkalinity and a loss with a decrease in alkalinity below that of the blood. The ventricular tonus may be affected, therefore, by alterations of the P_H of the perfusate independently of the effect of such changes upon the auricular output.

The effects of change of P_H upon the amplitude of contraction of the ventricle in the case of reversed sequence were not so marked as in hearts beating with normal rhythm. This would seem to support the idea again that an increase in ventricular amplitude observed, may be due largely to the increased intra-ventricular tension produced by an increase in auricular output.



A Showing reversed rythm

B 2:10 P_H 7.3

C 2:40 P_H 7.6

D 3:00 P_H 7.4

Fig. 3

DISCUSSION

The results of the present study, as presented above, would indicate that the heart of the terrapin is sensitive to relatively slight changes of H-ion concentration in the surrounding fluid. A decrease in acidity causes a prompt rise in tonus which is more evident in the auricles but is also present in the ventricle, and is accompanied by a falling off of the amplitude. An increase in acidity to a point slightly less alkaline than the blood produces a loss of tonus and an increase in amplitude of contraction in all chambers.

While the effect of auricular tone upon cardiac efficiency has been made clear through the work of Gesell, the question of the cause and control of such tone has been the subject of considerable controversy. Fano (11) reported in 1887 that strips of the turtle's auricle normally showed rhythmic tone changes. Botazzi (12) separates the movements of the auricular muscle of the turtle into two kinds and ascribes their independence to the structures of the myocardiac cells. To the sarcoplasm he attributes the function, in its tonic contraction, of supporting the anisotropic substance in its quicker contractions.

Botazzi and Grünbaum (13) point to the similarity existing between auricular tone changes and the contractions of smooth muscle. They contend, nevertheless, that tonus must be a function of the sarcoplasm and that the rapid clonic contractions of the auricular muscle which are superimposed upon the slower tone changes are due to the anisotropic substance of the fibrillae. (Smooth muscle, in their opinion, plays no important rôle in the rhythmic phenomena).

Rosenzweig (14) reports a layer of smooth muscle directly beneath the auricular endothelium in the turtle, which he regards as a continuation of the tunica media of the great veins, becoming thinner with distance from the mouth of the veins until only isolated fibers of smooth muscle are demonstrable in the ventricle. He attributes the tonic contractions of the auricles to this layer and the more rapid contractions to the cardiac muscle proper.

Botazzi (15), (16) observed that vagus stimulation produced rhythmic oscillations of tone in tone-free auricles. The vagus, therefore, was regarded as positively tonotropic. The sympathetic on the other hand caused oscillations to cease and generally reduced the tonus level. This nerve therefore was regarded as negatively tonotropic. Ouinuma (17) confirmed these observations.

The results of the work presented above seem to add one factor to the discussion. It is clearly shown in the preceding paragraphs that the ventricle exhibits changes in tone in response to chemical stimulation quite apart from any effect of the auricular beat upon the filling of the ventricle. With the data in hand it is quite impossible for us to account satisfactorily for this result either upon the sarcoplasm theory of Botazzi or upon the basis of the presence in the ventricle of isolated fibers of smooth muscle as reported by Rosenzweig.

CONCLUSIONS

1. Slight alterations in the P_H value of the perfusate within substantially normal limits, and in all cases upon the alkaline side of neutrality cause definite changes in the activity of the terrapin's heart.

2. A decrease in acidity to a P_H value of 7.6 to 7.8 causes marked changes in two phases of activity. The tonus especially in the auricles rises rapidly and the amplitude is decreased.

3. A slight raising of the P_H value to 7.3 increases the cardiac output in either or both of two ways, i.e., by a decrease in tone and hence an increase in initial volume, or by an increase in the amplitude of beat.

An experiment is reported showing that, in the case of a reversed sequence, tonus changes can be produced in the ventricle quite independently of any changes in the filling action of the auricular beat.

I wish to express my thanks to Dr. C. G. Rogers of Oberlin College, under whom the study was begun, and to Dr. Charles D. Snyder of the Department of Physiology of Johns Hopkins Medical School, for his kindness in allowing me space in his laboratory and for many helpful suggestions.

BIBLIOGRAPHY

(1) GASKELL: Journ. Physiol., 1880, iii, 57.
(2) MINES: Journ. Physiol., 1913, xlvi, 188.
(3) JERUSALEM AND STARLING: Journ. Physiol., 1910, xl, 279.
(4) KETCHAM, KING AND HOOKER: This Journal, 1912, xxxi, 64.
(5) MINES: Journ. Physiol., 1913, xlvi, 1.
(6) CLARK: Journ. Physiol., 1914, xlvii, 66.
(7) LEVY, ROWNTREE AND MARRIOTT: Arch. Int. Med., 1915, xvi, 389.
(8) GASKELL: Journ. Physiol., 1880, iii, 48.
(9) GESELL: This Journal, 1915, xxxviii, 404.
(10) GESELL: This Journal, 1916, xxxix, 239.
(11) FANO: Beitr. z. Physiol., Carl Ludwig, 1887, 297.
(12) BOTAZZI: Journ. Physiol., 1897, xxi, 1.
(13) BOTAZZI AND GRÜNBAUM: Journ. Physiol., 1899, xxiv, 50.
(14) ROSENZWEIG: Arch. f. Physiol., 1903, 192.
(15) BOTAZZI: Arch. Ital. d. Biol., 1900, xxxiv, 17.
(16) BOTAZZI: Arch. Ital. d. Biol., 1901, xxxvi, 277.
(17) OUINUMA: Pflüger's Arch., 1910, cxxxiii, 500.

A COMPARATIVE STUDY OF CARBON DIOXIDE PRODUCTION DURING STARVATION IN PLANARIA

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Earlier studies of the physiological condition of *Planaria dorotocephala* by the susceptibility method led to the conclusion that during starvation the rate of oxidation actually increases, so that an animal reduced from larger to smaller size by starvation is in almost the same metabolic condition as indicated by susceptibility to lethal concentrations of various agents, as a young fed animal of the same size (1), (4), (6, chap. vii). Between such animals, however, one difference was found to exist: the well-fed animal possesses a very much greater capacity than the starved animal for acclimation to various inhibiting agents in low concentrations and to other injurious conditions. When the starved animal is fed these differences disappear and its physiological condition is indistinguishable, so far as concerns susceptibility, from that of the well-fed, growing animal of about the same size. Even a single feeding after a period of starvation is sufficient to increase to a considerable extent the capacity for acclimation. If susceptibility relations are in any degree a measure of physiological condition, a period of starvation with the accompanying reduction in size, followed by resumption of feeding, brings about a process of rejuvenescence.

In connection with the earlier work a few estimations of carbon dioxide production were made by means of the Tashiro biometer, animals reduced by several months of starvation being compared with well-fed animals of the same size (4, p. 434). These estimations indicated that the CO_2 production of the starved, reduced animal was about equal to that of the well-fed, growing animal of the same size and much greater than that at the beginning of starvation. The biometer data agree in general with the susceptibility data but of course give no clue to the nature of the changes concerned in the increase of acclimation capacity on resumption of feeding.

During the last two years the earlier work has been supplemented by comparative colorimetric estimations of CO_2 production, by further work with the biometer, by determination of susceptibility to lack of oxygen in starved and fed animals and by repetition and extension of the experiments on susceptibility to chemical agents. The data on CO_2 production are presented below.

METHODS

The experiments are directly comparative in all cases: equal weights of the animals to be compared are placed in equal volumes of water containing the indicator in sealed tubes, the change of color with increasing acidity serving as a measure of CO_2 production.

Phenolsulphonephthalein, the indicator found to be most satisfactory, is used in a concentration of about 1/150,000 in water from the same source as that in which the stocks of worms are kept. Although the object of the experiments was the determination of the differences in CO_2 production rather than the actual amount of CO_2 produced, the experimental tubes were compared with the standard tubes prepared by Hynson, Westcott & Dunning with indicator in buffer solutions of different known pH, and the pH recorded.

That the increase in hydrogen ion concentration in the water in which the worms are kept is due to CO_2 and not to other acids is indicated by the fact that, on standing or shaking with air after removal of the worms, the original color of the indicator returns. The substance produced by the worms, which increased the hydrogen ion concentration, is therefore removed by this procedure and the probability that the animals secrete any other substance than CO_2 which acts as an acid in water and can be removed in this way is negligible, and other experiments show that they do produce considerable quantities of CO_2. The concentration of the indicator is the same in experimental and in buffer solutions. In this concentration, 1/150,000, and in fact in much higher concentrations this indicator is not appreciably toxic to planarians, and even in 1/10,000 the animals live indefinitely, though it acts as a weak inhibiting agent. The solutions of the buffer series differ by 0.1 pH, or at the ends of the series by 0.2 pH, but since color differences corresponding to far less than 0.1 pH are readily distinguishable with a little practice over those portions of the range where the indicator is most sensitive, the second decimal place is determined by estimation, or in some cases the sign + or − is added to the pH number of the color nearest the experimental color.

The well water used for experiment has a pH of 7.4 to 7.6 when first pumped, but on standing for some time or after five to ten minutes shaking with air pH = 7.6 to 7.8 or sometimes even 8.0. Since it seemed desirable in many cases to vary the pH of the starting point, the water was sometimes used at pH 7.6 to 7.7, while in other cases it was brought to 8.0 or 8.1 by shaking with air or by adding if necessary a few drops of sodium carbonate m/100.

The worms are weighed "dry", that is, with no excess of water. The procedure found most satisfactory is as follows: the worms to be weighed are dropped on filter paper from a pipette with as little water as possible and after about thirty seconds each animal is transferred to the container on the balance pan on the tip of the blade of a very narrow-bladed lancet which has been slightly vaselined to prevent as far as possible the adhesion of water. The container is a small glass vessel, e.g., the bottom cut from a small vial, and the worms are transferred to it by drawing the lancet blade carefully across its edge.

The container is of course balanced before the worms are added and the transfer and weighing of the worms must be done as rapidly as possible to avoid injury from drying, but since the total weight of even fifteen or twenty worms reduced by starvation is only a few milligrams, it must be reasonably accurate. After some practice it is possible to estimate rather closely the weight of a particular lot of worms and to reduce the time necessary for weighing by arranging the weights accordingly before the worms are transferred. Two minutes exposure to air which is not extremely dry will not usually injure the animals, but this is near the limit. As soon as weighed the worms are returned to water.

After approximately equal weights of the worms to be compared have been obtained, each lot is placed in a clean pyrex tube, rinsed twice in the indicator solution which is to be used, the tube is then filled with the solution and sealed.

Two methods of sealing have been used: first, that described by Haas (7), in which a tightly fitting paraffined rubber tube several centimeters long, attached to the open end of the pyrex tube, is closed by a clamp, after filling with the indicator solution to a level above the clamp to avoid air bubbles. This method has some disadvantages for motile animals such as Planaria, for some individuals may creep into the rubber tube, where they cannot be seen, and it is desirable to keep all in sight at all times in order to make certain that all remain in good condition and to determine whether unusual motor activity, which of course increases CO_2 production, occurs. Another method of sealing which has proved satisfactory consists in closing the tube first with a very tight plug of acid-free absorbent cotton soaked in the indicator solution, care being taken to exclude bubbles. This plug is pushed far enough into the tube to leave room for at least 1 cc. of solution above it and for a paraffine seal. A small bubble of air is left between the paraffine and the upper solution to prevent leakage from changes in pressure in consequence of slight temperature changes. Diffusion through the cotton plug is so slow that it is of no significance during the period of the experiment.

After the tubes are sealed they are of course kept under as nearly as possible the same conditions and from time to time the different lots of a series are compared directly with each other and with the standard tubes. The extreme range of color change used is from pH 7.6 to 8.1 at the beginning to pH 7.0 at the end, but in most cases only about half this range is used.

In order to avoid as far as possible errors resulting from differences in motor activity of the different lots, the heads are removed in almost all experiments one to twenty-four hours before the pH determinations. It has been found that except as regards motor activity, the removal of the head has very little or no appreciable effect on total CO_2 production. The headless animals are much less active than the normal, though of course able to react in the normal way when sufficiently stimulated. If the tubes are carefully handled and kept from sudden and great changes in light intensity, the headless animals usually remain motionless throughout the period of the experiment after the first few minutes.

Carbon dioxide production in relation to age. Since the present paper is a contribution to the question of the effect of starvation on the physiological age of the animals, it is necessary to present first some data concerning CO_2 production in well-fed animals of different physiological age, size being taken as a criterion of age in worms of a given stock (6, chap. iv). The following table gives a characteristic series.

TABLE 1

Series 666. Lot I, old animals. Lot II, young animals. Both well fed. In both heads removed

	LENGTH	NUMBER OF WORMS	WEIGHT	TIMES IN HOURS AND pH				
				0	2	3	5	6½
	mm.		*grams*					
I	25	3	0.0481	7.7	7.45	7.3+	7.25	7.2+
II	7	36	0.0470	7.7	7.3+	7.2	7.1+	7.05

Even though the thirty-six young weigh slightly less than the three old animals, they produce CO_2 much more rapidly. Similar comparisons of old and young animals have been repeated many times with worms of different sizes, with and without heads and by different persons, the experiment having been used as a laboratory experiment for students. The result is always the same, a higher CO_2 production per unit of weight in the young than in the old.

In *Planaria dorotocephala* the young worms of a stock are in all probability agamically produced, since gametic reproduction has never been observed in the locality from which the animals are collected (6, p. 386). In other species, however, the differences btween sexually produced young and old are like those between young and old of agamic origin and of the same sort as in *P. dorotocephala.*

Starvation stocks. It has long been known that Plan ria will live for several months without food and during this period will undergo reduction to a small fraction of its original size. The reduction in size is due to the fact that the animal is able to live upon its own structural substance after using up whatever nutritive reserves may be present. The alimentary tract is apparently the chief source of nutrition and undergoes reduction more rapidly than other organs of the asexual animals, but all parts of course undergo reduction more or less rapidly.

In making up starvation stocks careful standardization as regards several factors is necessary. In the first place, since differences in size, in nature or in a general laboratory stock represent differences in physiological age with corresponding differences in CO_2 production, animals of as nearly as possible the same size must be selected for a starvation stock. Second, these animals must be as nearly as possible in the same nutritive condition, for CO_2 production varies greatly with nutritive condition. Third, the larger animals consist physiologically of more than one zoöid (2), (3), (6, chap. vi) and almost invariably undergo fission during the first few days of starvation at a level slightly posterior to the mouth (*aa*, fig. 1). The anterior and posterior products of fission undergo regulation into normal animals (fig. 2, anterior; fig. 3, posterior) and these two animals resulting from fission, the "anterior" and "posterior" animals as they may for convenience be called, show characteristic differences in body-form, rate of reduction and physiological condition during starvation, and the posterior animal often undergoes a second fission and regulation.

These differences between anterior and posterior animals must be briefly considered. The process of regulation in the anterior product of

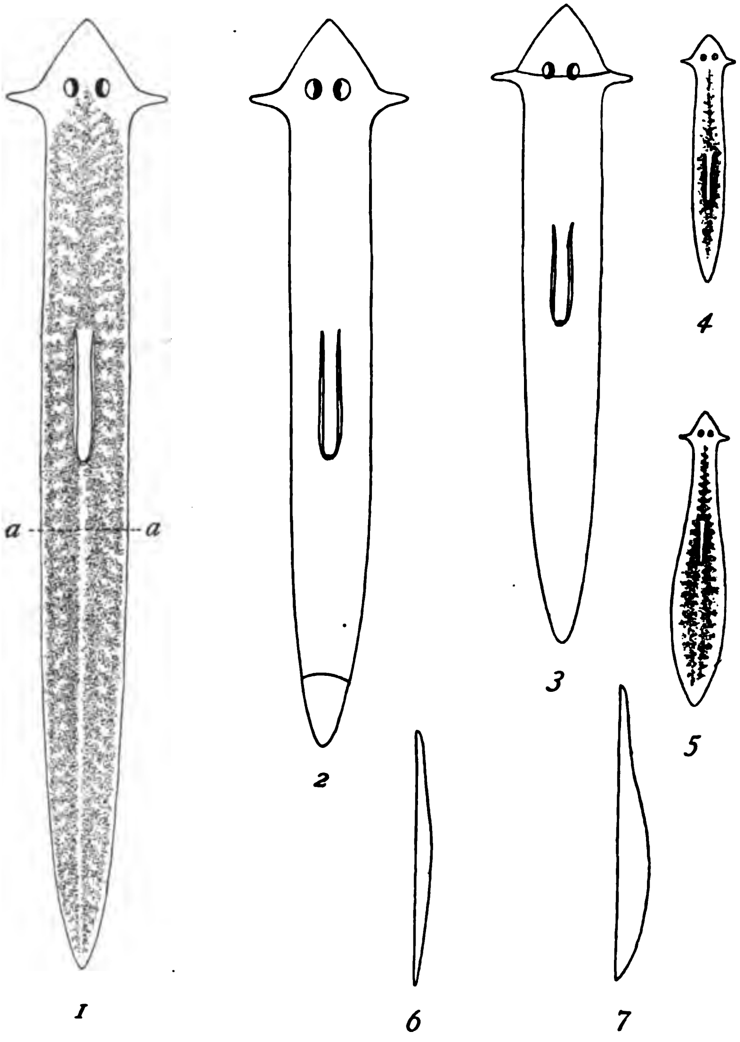

Fig. 1. A large, well-fed individual of *Planaria dorotocephala*, showing alimentary tract: *aa*, level at or near which fission usually occurs.

Fig. 2. Anterior animal resulting from fission

Fig. 3. Posterior animal resulting from fission.

Fig. 4. Anterior animal reduced by about three months of starvation, showing body-form and extent of alimentary tract.

Fig. 5. Posterior animal reduced by about three months of starvation, showing body-form and extent of alimentary tract.

Fig. 6. Outline of starved anterior animal in side-view.

Fig. 7. Outline of starved posterior animal in side-view.

fission consists chiefly in the development and compensatory growth of a new postpharyngeal region (fig. 2), while in the posterior product it consists in the development and growth of a new head, prepharyngeal and pharyngeal region (fig. 3). The anterior animal is physiologically a single individual, except the extreme posterior end, where a second zoöid is usually physiologically distinguishable. The postpharyngeal region of a large worm like that in figure 1 always consists of two or more zoöids (2), (3), (6, chap. vi), and the posterior animal resulting from the fission of such a worm therefore consists of at least two zoöids of nearly equal length. In the anterior animal then practically the whole body is under the control of the dominant head and behaves as a well-coördinated whole, while the two zoöids composing the posterior animal show a certain degree of independence, which becomes more and more evident as starvation progresses.

The alimentary tract of the large, well-fed animal fills most of the body (fig. 1) and since it is not only a digestive organ but the chief place of storage of nutritive reserves, its cells are large and loaded with fat and other substances. Moreover, the bulk of the alimentary tract is greater in the postpharyngeal than in the prepharyngeal region, as figure 1 shows. In relation to its size, then, the posterior animal has a larger volume of nutritive reserves and entodermal tissue as a source of nutrition during starvation than the anterior animal.

These two differences between anterior and posterior animals arising by fission at the beginning of starvation, viz., the less complete coördination and the greater volume of the alimentary tract in the posterior than in the anterior animal, determine certain differences in the course and rate of reduction during starvation. After some three months of starvation (more or less according to temperature) the anterior animal presents the appearance of figure 4, the posterior animal that of figure 5. In the former the head is relatively large, the body slender and of almost uniform width throughout and the alimentary tract has undergone great reduction, all the more lateral portions having completely disappeared and the anterior and posterior terminal portions being reduced in length and diameter. In such animals the spaces in the parenchyma formerly occupied by parts of the alimentary tract are clearly visible in the lateral and terminal body-regions when the worms are brought under slight pressure. In the posterior animal the head is relatively small, the prepharyngeal region slender and the postpharyngeal region much broader, and the alimentary tract is much more reduced in the prepharyngeal than in the postpharyngeal region. Figures 6 and 7 are outlines of the two animals in side-view to show the differences in the dorso-ventral direction. The anterior animal (fig. 6) is thin throughout, the thickness decreasing in both directions from the pharynx, while in the posterior animal (fig. 7) the anterior zoöid is thin but the posterior zoöid is much thicker. Moreover in these advanced stages of starvation it can be clearly seen that in the anterior animal the whole body is well coördinated while the broad, thick posterior zoöid of the posterior animal is dragged about by the anterior zoöid as a more or less passive mass, although with strong stimulation it may be brought under control.

In the anterior animal starvation and reduction have evidently progressed more rapidly than in the posterior animal as a whole, although in the latter the anterior portion, i.e., the anterior zoöid, being the more active, has undergone reduction to a greater extent than the posterior. The total bulk of alimentary tract in the posterior animal (fig. 5) is much greater than in the anterior animal (fig. 4). If the rate of CO_2 production changes during starvation, we should expect to find the change occurring more rapidly in the anterior than in the posterior animal, and this is actually the case, as will appear below.

In most cases, however, the posterior animal itself undergoes fission and regulation during the first few weeks of starvation, giving rise to two individuals, "anterior-of-posterior" and "posterior-of-posterior," which show essentially the same differences as the anterior and posterior products of the first fission but are of course smaller. Much less frequently anterior animals undergo fission during starvation and in this case the two products, "anterior-of-anterior" and "posterior-of-anterior," are much alike in size and shape except that the regenerated head of the posterior-of-anterior animal always remains smaller than the head of the anterior-of-anterior animal, which is the original head.

Such products of second fission use their body-substance not only in functional activity and partial maintenance of certain organs, but also in regulation and growth of new parts, consequently a second fission during starvation must accelerate the progress of starvation.

In addition to the differences in size due to fission, differences in size which may be called accidental appear in starvation stocks. Of two animals of the same size at the beginning of starvation one may contain a larger amount of nutritive reserves and therefore undergo reduction more slowly than another. Sometimes also one animal eats another and thus obtains sufficient food to delay starvation and reduction to a considerable degree. This cannibalism occurs chiefly where animals of very different size are kept together in the more advanced stages of starvation, but injured animals and those with dying and disintegrating parts are often attacked and eaten. It is prevented to a large extent by avoiding over-crowding, by isolating animals of different sizes and by removing dying or injured animals as far as possible.

In order to determine what changes occur during starvation, it is necessary not only to standardize the stock at the beginning as regards size, nutritive condition and preceding fissions, but also to distinguish between the different products of first and second fissions or, where this is not possible with certainty, as is often the case in the two products of fission of anterior animals during starvation, to use size as a criterion of stage of starvation. If anterior and posterior, or anterior of posterior and posterior of posterior, or animals of very different size are mixed in the lots used for comparison, the results may be entirely misleading. Since starvation proceeds more rapidly at higher than at lower temperature, stocks to be compared must be kept at the same temperatures.

In all the following experiments all these points have received attention. All starvation stocks were made by selecting animals of the same size from one general stock which has been fed in the laboratory three times a week with beef liver during at least two months. Some stocks were composed of anterior or of posterior animals from fissions which had occurred within a few days preceding selection. In others animals like that of figure 1, 25 mm. in length, were selected and either allowed to undergo fission during the first few days of starvation as almost every individual does, or each animal was cut approximately at the fission plane (*aa*, fig. 1) and the anterior and posterior animals resulting were used as starvation stocks. In short, each lot of animals in each experiment is as nearly uniform in all these respects as it was possible to make it.

For long periods of starvation, large physiologically old animals were used, since they undergo more extreme reduction before becoming too small to handle readily, but it has been found that small, young animals reach essentially the same physiological condition after a short starvation period as large, old animals, after a much longer starvation period. In order that animals in different stages of starvation may be available for comparison, starvation stocks are made up at intervals of a few days or a few weeks and kept as nearly as possible under identical conditions.

The earlier stages of starvation. The stocks used for study of the earlier stages of starvation consisted of anterior animals 12 to 14 mm. in length, the starvation period beginning some ten days after fission.

In such animals the regeneration at the posterior end is about completed but the posterior zoöid is not far enough developed for the occurrence of another fission during the first weeks of starvation. The data for five characteristic series are tabulated in table 2.

Table 2 shows the effect of a few days of starvation upon the CO_2 production. In each series animals fed within one or two days (I) are compared with animals starved from three to twelve days (II, III). In the first three series of the table heads were removed before comparison, in the other two the animals were intact. The fourth column, "No. of worms," shows that the starved worms have lost somewhat in weight: in series 656 II, the number 5⅓ in this column means that the anterior third of a worm was added to the five whole worms to make up the weight. In the columns under "times of record and pH" the pH as observed at different times is given. Records are given only up to seven and one-half hours, but most of the series were continued several hours more, usually at least ten hours in all and in some series eighteen hours. The later readings of pH, however, do not in any case alter the result except that the differences between the two lots compared increase as time goes on.

Although in every series the weight of the starved worms is greater than that of the fed worms, the starved worms always produce less CO_2 than the fed worms. Moreover, in series 643, lot III, with seven days of starvation produces less CO_2 than lot II with three days of starvation. These experiments with short periods of starvation have been repeated frequently and have been used as class experiments with the same result in all cases. There can be no doubt that the total CO_2 production of these animals decreases rapidly during the first few days of starvation.

The later stages of starvation: anterior animals. After the rapid decrease in CO_2 production during the first few days of starvation, there is a period of at least several weeks during which a slight further decrease occurs, but sooner or later an increase appears. This increase appears earlier at higher than at lower temperatures, earlier in anterior than in posterior animals and earlier in animals which have undergone a second fission and regulation than in those which have undergone fission and regulation only once. The data for anterior animals follow in table 3.

In table 3 lot I is in all cases the earlier, lot II, the later stage of starvation. The table gives the number of days since last feeding, the length of the worms at the time of pH determination, the number of worms in each lot, the total weight of each lot, the number of comparative deter-

TABLE 2

Early stages of starvation

SERIES	DAYS SINCE LAST FEEDING	HEADS	NUMBER OF WORMS	WEIGHT	TIMES OF RECORD IN HOURS AND pH										
					0	1½	2	2½	3	3½	4	5	5½	6	7½
				grams											
654A I	1	Absent	4	0.0237	8.0		7.8−			7.5+				7.4−	
654A II	8	Absent	5	0.0271	8.0		7.8			7.6+				7.4+	
655 I	2	Absent	4	0.0257	8.0		7.8						7.5−		
655 II	9	Absent	5	0.027	8.0		7.9						7.6−		
656 I	1	Absent	4	0.0277	8.0			7.7			7.5				7.3−
656 II	12	Absent	5½	0.0288	8.0			7.8−			7.6				7.4
643 I	½	Present	4	0.0283	8.0	7.7+			7.6−			7.4			
643 II	3	Present	4	0.0284	8.0	7.8−			7.6			7.4+			
643 III	7	Present	5	0.0293	8.0	7.8			7.6+			7.5			
642 I	1	Present	4	0.0267	8.0		7.8+			7.7		7.6−			7.4−
642 II	6	Present	5	0.0269	8.0		7.9			7.8		7.7			7.5

minations made in each series and the pH readings for several hours. The color comparisons were made with great care, the two lots of each series being compared both with each other and with the standard tubes of the colorimeter in uniform light. Where repeated comparisons were made with the same lots, clean tubes and fresh indicator solution were of course used, and a different pH was used as starting point in at least one of the repetitions in each series, as the table shows (see pH under 0 hours). The second decimal place in the pH readings is estimated, not exact, and serves chiefly to indicate whether the difference

TABLE 3

Anterior animals 12 to 13 mm. at beginning of starvation. Heads removed before weighing

SERIES		DAYS SINCE FED	LENGTH	NUMBER OF WORMS	WEIGHT	COMPARISONS WITH SAME LOTS		TIMES OF RECORD IN HOURS AND pH					
								0	1	2	3	4	5
			mm.		*grams*								
699	I	15	11–12	4	0.0232	First	I	8.1		7.9	7.7+	7.5	
	II	77	8	9	0.0221		II	8.1		7.95	7.8+	7.7+	
						Second	I	7.6	7.5−			7.3	
							II	7.6	7.5			7.4−	
699A	I	38	9	4	0.0156			7.7	7.63		7.53	7.45	7.38
	II	100	6	9	0.0163			7.7	7.6		7.5	7.4	7.33
705						First	I	7.8	7.7+	7.62			
							II	7.8	7.7	7.58			
	I	36	8–9	4	0.0186	Second	I	7.7	7.65	7.55		7.38	7.33
	II	98	6	9	0.0187		II	7.7	7.65−	7.53		7.36	7.3
						Third	I	7.7	7.62	7.55	7.48		
							II	7.7	7.6	7.53	7.45		
713						First	I	7.7	7.65	7.58			
							II	7.7	7.62	7.55			
	I	57	7–8	3	0.0119	Second	I	7.7		7.6		7.5	7.45
	II	119	5	7	0.0122		II	7.7		7.6		7.48	7.43
						Third	I	7.6	7.5	7.44	7.4	7.36	
							II	7.6	7.5	7.42	7.38	7.34	

between the two lots of a series is great or small, but in no case was a difference recorded unless it was perfectly distinct and observed in all of several comparisons made within two or three minutes with the tubes in different relative positions. Many of the records were confirmed independently by other persons. The transfer of the worms individually in weighing makes the use of small numbers desirable in order to avoid long exposure of some animals to the air, and this of course makes the differences in pH smaller than they would be if large numbers were used.

In series 699 of the table, lot I with fifteen days starvation produced CO_2 more rapidly than lot II with seventy-seven days of starvation in both of the two tests made. After these tests the worms of these two lots were returned to water and allowed to starve for twenty-three days more, during which time they regenerated heads in place of those cut off. At the end of this time the heads were removed again, the lots weighed and another pH determination made, the data for which are given in series 699A. At this time the later stage of starvation (lot II) produces CO_2 more rapidly than the earlier (lot I), i.e., the relative rates are the reverse of those in series 699. Moreover, taking into account the loss of nearly one-third in weight between the time of series 699 and 699A, the change in pH, though not directly comparable in the two series because of different starting points, indicates that the rate of CO_2 production of lot II is higher in 699A than in 699. These two series, which show earlier and later conditions in the same two lots, afford evidence in two ways that the rate of CO_2 production increases during the later stages of starvation. The great loss of weight during the twenty-three days between the two series is of course due not merely to starvation, but also to the fact that during this period the animals regenerated new heads, which were removed before the second weighing.

In series 705, lot I, starved thirty-six days, shows less rapid change in pH than lot II, starved ninety-eight days, all three tests giving the same comparative results. In series 713 still more advanced stages of starvation are used, lot I, fifty-seven days, lot II, one hundred and nineteen days, and again the more advanced stage shows the more rapid change in pH in all three tests.

From other starvation stocks started at other times and from other general stocks of worms three series of comparative pH determinations like those of table 3 were made, the starvation periods being thirty-nine and seventy-eight, forty and seventy-nine, forty-three and eighty-two days. In two of these series the more advanced stage showed the more rapid change in pH, in one a slightly less rapid change than the less advanced stage. In these series the heads were not removed and it is possible that differences in motor activity may have been responsible for the exceptional result in the one series. Unfortunately it was impossible to carry on to later stages the stock from which these series were taken.

The more important data on anterior animals are briefly summarized as follows: A total of thirty-one animals, starved thirty-eight to fifty-seven days, was compared in lots of equal or nearly equal weight with a total of seventy animals, originally the same size, but starved seventy-seven to one hundred and nineteen days. Ten comparative pH determinations were made with six pairs of lots, each determination comprising two or more pH records at intervals of one to three hours. In nine of these determinations (25 and 55 animals) the more advanced stage of starvation showed a distinctly more rapid change in pH. In one determination (6 and 15 animals) the more advanced stage showed a slightly less rapid change in pH, but in this series the starvation periods were such that no very great differences were to be expected and since the heads were present differences in motor activity may have affected the result. No other exceptional cases have been observed with starvation periods of forty and eighty days or more in the two lots.

The later stages of starvation: posterior animals and products of second fission. Most of the posterior animals resulting from the first fission or section (p. 239) at the beginning of starvation undergo a second fission and regulation during the first few weeks of starvation, so that in the later stages of a starvation stock, instead of posterior animals, one finds mostly anterior-of-posterior and posterior-of-posterior animals (p. 238). Sufficient material has, however, been found in my stocks for four comparative series of earlier and later stages with posterior animals, though none represent very late stages of starvation. In these series lot I, six worms in each series, a total of twenty-four worms, had been starved thirty-nine to forty-three days and lot II, eleven, eleven, nine and ten worms, a total of forty-one worms, had been starved seventy-eight to eighty-two days, i.e., about twice as long as lot I. In each series the earlier stage, lot I, showed a more rapid change in pH than the later stage, though in one series the difference was very slight. In these posterior animals then the rate of CO_2 production is still higher in the earlier than in the later stage after these starvation periods, while in anterior animals (see preceding section) with corresponding starvation periods, the rate of CO_2 production is almost always higher in the later than in the earlier stage.

For the still more advanced stages of starvation it was necessary to use the products of the second fission of posterior animals. These of course have used a part of their body substance in a second regulation as well as in starvation, consequently at any given time the products of the second fission of posterior animals represent a more advanced stage of starvation than animals which have starved for the same length of time, but have not undergone a second fission and regulation. The chief data obtained with these groups of animals are given in table 4.

Table 4 is similar in plan to table 3, except as regards the second column, in which the origin of the animals of each lot as regards preceding fissions is given. In series 714 and 700 posterior animals (I) are compared with posterior products of a second fission (II, posterior-of-posterior), the period of starvation of both lots being in series 714, fifty-nine days, in series 700, seventy-eight days. In both series, lot II, the posterior-of-posterior animals, shows the more rapid change in pH. In series 714 four comparisons were made, but since the result was the same in all only two are given in the table. In series 700 only two comparisons were made. Series 697 gives data for posterior (I) and posterior-of-posterior animals (II), starved seventy-seven days, the latter showing the more rapid change in pH.

Series 706 and 712 are both comparisons of posterior-of-posterior animals in earlier (I) and later stages (II) of starvation. In series 706, where the two lots have been starved respectively thirty-seven and ninety-nine days there is no difference in the first comparison and a very slight difference in the second. In series 712 with fifty-seven and one hundred and nineteen days of starvation the later stage shows the higher rate of change in pH in all three comparisons.

This table shows then, first, that after two months or more of starvation, products of a second fission of posterior animals show a higher rate of CO_2 production than the posterior animals themselves; and second, that posterior products of second fission (posterior-of-posterior) in sufficiently advanced stages of starvation possess a higher rate of CO_2 production than in earlier stages.

In another series not tabulated anterior-of-posterior animals were compared at twenty-three and eighty-five days of starvation, the earlier stage showing a very slightly more rapid change in pH than the later. Heads were not removed in this case and it was recorded at the time of experiment that the earlier stage showed distinctly more motor activity

TABLE 4

Posterior animals and products of fission of posterior animals: length at beginning of starvation 12 to 13 mm.; heads removed before weighing except in series 700

SERIES		ORIGIN	DAYS SINCE FED	LENGTH	NUMBER OF WORMS	WEIGHT	COMPARISONS WITH SAME LOTS		TIMES OF RECORD IN HOURS AND pH 0	1	2	3	4	5	6	7
				mm.		grams										
714	I	Post.	59	9–10	3	0.0123	First	I	7.6	7.55			7.45			7.38
	II	Ant.-of-post.	59	5–6	13	0.0124		II	7.6	7.55			7.43			7.35
							Second	I	7.6				7.44	7.4		
								II	7.6				7.42	7.38		
700	I	Post.	78	8–9	4	0.0136	First	I	7.6	7.5		7.42		7.35		
	II	Ant.-of-post.	78	5–6	11	0.0138		II	7.6	7.5–		7.37		7.3		
							Second	I	7.8	7.75				7.6		
								II	7.8	7.73				7.52		
697	I	Post.	77	8–9	4	0.0134	First	I	8.1	8.1–			7.95		7.9	
	II	Post.-of-post.	77	6–7	10	0.0131		II	8.1	8.0+			7.9		7.8	
							Second	I	7.6	7.58				7.4	7.37	
								II	7.6	7.55				7.37	7.33	
706	I	Post.-of-post.	37	8	4	0.0136	First	I	7.7	7.6+			7.38	7.32		
	II	Post.-of-post.	99	6	10	0.0130		II	7.7	7.6+			7.38	7.32		
							Second	I	7.7	7.65			7.49			
								II	7.7	7.65+			7.5			
							First	I	7.7	7.6	7.55					
								II	7.7	7.57	7.5					
712	I	Post.-of-post.	57	7–8	4	0.0127	Second	I	7.7		7.6			7.48		
	II	Post.-of-post.	119	5	10	0.0124		II	7.7		7.6			7.45		
							Third	I	7.6	7.52		7.48	7.38			
								II	7.6	7.52		7.42	7.30			

than the later, consequently the result is of doubtful value. No other exceptions have been found.

Miscellaneous data. From other starvation stocks starved at a higher temperature and therefore more rapidly than those used in the preceding series, anterior animals, 10 to 11 mm. in length after twelve days of starvation showed a markedly lower rate of CO_2 production than anterior animals (or perhaps products of second fission of anterior animals) 5 to 7 mm., after forty-seven days of starvation. In another series from a stock starved forty-two days, the largest anterior animals (8 mm.) showed a lower rate of CO_2 production than the smallest anterior animals (5 to 6 mm., perhaps products of second fission). Here the smaller animals, either because of a second fission and regulation or because of accidental conditions (p. 238) represented a more advanced stage of starvation showed a higher rate of CO_2 production than the larger.

After eighty days of starvation anterior animals show a distinctly higher rate of CO_2 production than posterior animals with the same starvation period. In view of the data already presented this difference is to be expected from the fact that anterior animals undergo more rapid reduction than posterior animals.

In still another experiment anterior and posterior animals from a stock starved forty-three days were cut in two at the mouth and equal weights of the posterior pieces of each were compared. The posterior region of the anterior animals has of course been concerned in the formation of a new posterior end since starvation began, while that of the posterior animal has not. It was also noted above (p. 237) that the anterior animal undergoes reduction more rapidly than the posterior in starvation, and figures 4 (anterior) and 5 (posterior) indicate the difference in intestinal volume of the posterior region of the two animals. The posterior pieces of the anterior animals showed a distinctly more rapid change in pH than the posterior pieces of the posterior animals. In other words, the pieces in which the alimentary tract was most reduced showed the higher rate of CO_2 production, although starved for the same number of days as the other pieces.

In one series anterior animals starved one hundred and thirty-four days, without fission during starvation, showed a less rapid change in pH than posterior-of-posterior animals starved seventy-two days, both lots being about 6 mm. in length. In this case the anterior animals had been in the biometer (see p. 250) preceding the colorimetric test and may have been injured by exposure to the air, or possibly by mercury vapor, such injury having been observed in other cases in the biometer. This is the only exceptional case in this group of data, and it is at least of doubtful value.

Starved and fed animals. The chief purpose of the present paper is to present the data concerning the conditions during starvation. Some comparative work has been done on CO_2 production in starved and fed animals and on the effect of feeding after periods of starvation, but the data are not yet as complete as is desired, and their presentation in full is postponed to another time. A few characteristic results among those already obtained along this line are briefly stated to show what has already been determined along this line, and as a basis for discussion of certain questions.

Direct comparison of pH change in the advanced stages of starvation with that of large, well-fed animals by the method described above presents difficulties because the large number of starved animals necessary to equal in weight even one or two of the large ones cannot be handled rapidly enough in weighing to avoïd danger from drying. Other methods of procedure are more satisfactory. A few direct comparative experiments have, however, been made in which pieces of the large animals including the region between head and mouth, the head being removed, have been used instead of the whole animals. Animals starved one hundred and twenty-three days and reduced by starvation and fission from 25 mm. to 4 or 5 mm. produce CO_2 more rapidly than pieces of well-fed animals of the original length, i.e., 25 mm. and at about the same rate as pieces of well-fed animals 15 to 16 mm. which show a somewhat higher rate than the pieces of 25 mm. animals. Anterior animals from another stock, starved one hundred and thirty-seven days without fission during starvation, and reduced from 15 mm. to 4 or 5 mm., showed in one series a distinctly higher rate than pieces from 25 mm. well-fed animals, while in another series where only a single piece of an old animal and three starved worms were used, the rate was somewhat lower in the starved animals than in the fed piece. In general the data indicate that after four months or more of starvation, the rate of CO_2 production is actually higher than, or at least about equal to the rate at the beginning of starvation, i.e., the decrease in rate during the early stages of starvation has been balanced or more than balanced by an increase in rate during the later stages. This result agrees with and confirms the data of preceding sections, which show an increase in rate in the later stages of starvation.

It is a familiar fact that planarians which have been reduced by starvation to a small fraction of their original size, will begin to grow again when fed and may again attain the original size and again be reduced by starvation. So far as our knowledge goes, this alternation of growth and reduction may be repeated indefinitely with proper food conditions. According to criteria thus far applied, animals which are fed after a period of starvation and reduction are physiologically younger than they were at the beginning of starvation and are comparable to well-fed animals of the same size as themselves, as regards bodily proportions, rate of growth, susceptibility to various agents, capacity for acclimation (4), (6, chap. vii). As feeding is continued and increase in size again occurs, rate of growth, susceptibility and capacity for acclimation undergo gradual decrease, as in other animals undergoing growth and physiological senescence, so that when the animals again attain the original length, e.g., 25 mm., their condition in these respects is not appreciably different from the condition before starvation. The data at hand concerning the effect on CO_2 production of feeding after starvation confirm the earlier conclusions.

It was shown in table 2 that even a few days of starvation brings about a marked decrease in CO_2 production. It has been found that a single feeding after such a short period of starvation brings the animal back almost to its original condition, and that after two or three feedings at intervals of a day or two its rate of CO_2 production is the same as or slightly higher than that of fed animals of the same size. For example, animals starved eleven days and then fed once with liver twenty-four hours before comparison, show a rate almost as high as that of animals of the same size fed three times a week, and very much higher than that of animals starved twelve days. A second feeding twenty-four to forty-eight hours after the first is usually sufficient to establish the original rate.

After longer periods of starvation more food is required to raise the rate of CO_2 production to the level of that of well-fed animals of the same size. For example, animals starved eighty-seven days and reduced by starvation, fission and regulation from 25 mm. to 4 or 6 mm., after feeding three times on three successive days, show a rate of change in pH almost midway between that of young well-fed animals 5 to 6 mm. and animals 4 to 6 mm. starved ninety days without feeding. Animals starved eighty-seven days and reduced from 25 mm. to 4 or 6 mm., after feeding seven times in eight days show a rate of CO_2 production as high as, or slightly higher than that of young, well-fed animals of the same size. Three feedings are not sufficient to bring these animals up to the level of young, well-fed animals of the same size, but seven feedings accomplish the result and if feeding is continued, the animals from this time on are indistinguishable in any way, so far as we know at present, from the young, growing animals, which have not been starved. As regards rate of CO_2 production as well as other physiological characteristics, the animals which have been starved and then fed have undergone a physiological rejuvenescence, the degree of rejuvenescence depending on and varying directly with the degree of starvation and reduction.

Comparative CO_2 estimations with the Tashiro biometer. In the earlier work on starvation in Planaria a few data obtained with the Tashiro biometer (10, Appendix) were presented (4, p. 434). According to these data, animals starved ninety-four days showed a rate of CO_2 production about equal to that of young fed animals of the same size.

These earlier data have now been supplemented by some further work with the same apparatus. In these later estimations single headless pieces from the anterior body region of well-fed worms 25 mm. in length were compared with an approximately equal weight of headless animals reduced by starvation, fission and regulation from 25 to 6 or 7 mm. In ten estimations with differences in weight between fed and starved not exceeding 14 per cent with the excess in different sides in different cases, the starved animals showed in every case a distinctly higher rate of CO_2 production than the old fed pieces.

Several comparisons between animals starved one hundred and twenty to one hundred and thirty days and young fed animals of the same size gave a higher rate of CO_2 production for the starved than for the fed animals. These results do not agree with those of the colorimetric determinations.

Work with the biometer was discontinued because I came to believe that the method was not suitable for this material. The animals are more or less stimulated by handling and the starved animals are usually distinctly more susceptible than the fed and react more strongly to such stimulation. With the biometer it is necessary to make the estimations as soon as possible, i.e., within five or ten minutes after the animals are placed in the chambers, because even the headless animals usually become restless after a time on account of exposure to the air, although the atmosphere of the chambers is saturated with water vapor, or for some other unascertained reason. Consequently the carbon dioxide estimation is made before the animals have recovered from the stimulation due to the transfer and since there is every indication that the starved animals are more stimulated than the fed the biometer is likely to show too high a rate of CO_2 production for the starved animals. That this is the case was proved for some series by making a colorimetric comparison with the animals used in the biometer. Starved animals which showed in the biometer a higher rate than the fed showed a rate equal to or lower than that of the fed animals by the colorimetric method. With the latter method the determination extends over several hours and the headless animals usually do not move at all after the first few moments, if the tubes are properly handled. There is good reason to believe, therefore, that the colorimetric data are more nearly correct than those obtained with the biometer, but if the rates as shown in the biometer result from a greater degree of stimulation of the starved animals the reason for their greater sensitiveness or more intense reaction remains to be explained. The following discussion will throw some light on this point.

DISCUSSION

The colorimetric determinations indicate that the rate of CO_2 production falls rapidly and to a marked degree during the first few days of starvation, then more slowly during several weeks, and that this decrease is followed sooner or later by an increase in rate which may balance or even overbalance the earlier decrease, so that in advanced stages of starvation the rate of CO_2 production may be as high as, or higher than that at the beginning of starvation. That the condition of the alimentary tract is an important factor in these changes is indicated by several facts: first, the rapid decrease during the first few days of starvation can be compensated and the animal brought back to the original level by a single feeding; second, the decrease in rate apparently continues as long as any appreciable amount of nutritive reserve is present in the cells of the alimentary tract, while in the period of increase in rate the animal is apparently subsisting entirely upon its own protoplasm and the alimentary tract is reduced more rapidly than the body as a whole; third, in animals starved for the same length of time, those in which the alimentary tract is most reduced show in general the higher rate of CO_2 production; fourth, even after several months of starvation, a few feedings bring the rate of CO_2 production up to that of young fed animals of the same size as the starved animals, i.e., to a level far above that at the beginning of starvation. The metabolism of the alimentary tract evidently plays a large rôle in the total CO_2 production of well-fed animals, but it is also evident that this metabolism decreases rapidly in the absence of food and increases again when food is given. This is to be expected since the alimentary tract is functionally concerned with food, and in the absence of food its functional activity must decrease. Undoubtedly some functional activity is concerned in the transformation and removal of the nutritive reserves from the cells of the alimentary tract, but as starvation progresses and the reserves gradually disappear, even this decreases and sooner or later the alimentary tract becomes a practically functionless, quiescent organ and under these conditions autolysis in its cells probably increases and it becomes itself a source of nutrition for other parts.

The other essential organs of the body, ectoderm, muscles, nervous system, etc., do not undergo any such loss of function in starvation as does the alimentary tract. Their functional activity is maintained or even increased, since the starved animal becomes more active. All these facts indicate that the decrease in CO_2 production during starvation in Planaria is at least in large measure the result of a decrease in the metabolic activity of the alimentary tract. Probably the period of most rapid decrease at the beginning of starvation coincides with the period of disappearance of food from the tract, and the later period of slow decrease with the period of mobilization of reserves.

The increase in rate of CO_2 production during the later stages of starvation and the fact that starvation followed by feeding accomplishes a rejuvenescence as regards rate of CO_2 production and various other characteristics, remain to be accounted for. It is in this connection that the significance of the earlier work on susceptibility in relation to starvation appears (4), (6, chap. vii). These earlier investigations showed that the susceptibility of Planaria to lethal concentrations of KNC and various other toxic agents increases from the beginning of starvation and that animals reduced by starvation show about the same or a somewhat higher susceptibility than fed animals of the same size. It was found, however, that the capacity for acclimation decreases during starvation but is increased above the original capacity by feeding after starvation. The conclusion drawn from these earlier experiments was that the rate of oxidation underwent an increase during starvation so that the animals became physiologically younger in this respect as starvation progressed, but since the capacity for acclimation decreases during starvation, while it is greater in young than in old animals, it was suggested that nutritive condition as well as rate of oxidation is a factor in acclimation, and that the process of rejuvenescence is not complete in all respects until after the starved animal has been fed.

Although the same general conclusion, viz., that starvation followed by feeding accomplishes a process of rejuvenescence is drawn from the data on susceptibility and those on CO_2 production, an apparent discrepancy exists in the data obtained by the two methods concerning what occurs during starvation. Susceptibility increases from the beginning of starvation, and at the end of a starvation period is at least equal to that of fed animals of the same size as the starved, and is not further increased by feeding, while CO_2 production decreases, at first rapidly, then more slowly and sooner or later begins to increase, but is increased still further by feeding after starvation.

This apparent discrepancy in the results of the two methods depends, at least very largely, upon the fact that in the earlier experiments susceptibility was always determined by survival time of the ectoderm and body wall, no account being taken of the condition of the alimentary tract, while the data on CO_2 production concern total CO_2 production, that of the alimentary tract as well as that of the ectoderm and body wall. In the earlier work on susceptibility it was observed that in lethal concentrations the alimentary tract of fed animals disintegrated almost as early as ectoderm and body wall, while in starved animals the alimentary tract might remain completely intact for some time—depending on the concentration—after ectoderm and body wall were completely disintegrated or cytolyzed. The significance of these facts was not recognized until the work on CO_2 production made it clear. Since then the experiments on susceptibility have been repeated many times, not only with KNC but with various other agents. This later work shows that the susceptibility of ectoderm and body wall increases from the beginning of starvation, while the susceptibility of the alimentary tract, not only as compared with that of the body wall, but as compared with that of the alimentary tract at the beginning of starvation decreases during the earlier period of starvation, but in the advanced stages increases, though it remains below that of the body wall until the animals are fed, when it soon attains the relation characteristic of fed animals. These experiments were made with intact animals, where the chemical agents must pass through the body wall to reach the alimentary tract, but they show the changes in relative susceptibility of tract and body wall and the differences in susceptibility of the tract at different times. Where directly exposed at cut surfaces, the functionally active alimentary tract of fed animals is fully as susceptible as the body wall, that of starved animals less susceptible than the body wall.

It is evident then that when alimentary tract as well as body wall is taken into account in determining susceptibility, the data on susceptibility and those on CO_2 production are not in conflict, but supplement and confirm each other. Taking into account all the facts at present available, the following interpretation suggests itself: the ectoderm and body wall, which maintain their functional activity and subsist in part upon their own substance from the beginning, show a gradual increase in rate of oxidation during starvation, while the rate of oxidation in the alimentary tract undergoes rapid and marked decrease in the absence of food and probably still further decrease more slowly, as the reserves are used. Alimentary tract oxidation in well-fed animals is so large a part of total oxidation that the decrease in activity of the alimentary tract in the absence of food brings about a decrease in total CO_2 production, in spite of the probable increase in CO_2 production in ectoderm and body wall. Gradually, however, the alimentary tract approaches a minimum of oxidative activity, and as its substance is used, its rate of CO_2 production also begins to rise. Somewhere in the course of these changes increase in total CO_2 production begins to appear. In advanced stages of starvation the ectoderm and body wall are functionally active, while the alimentary tract is quiescent, consequently the animal is younger as regards susceptibility of ectoderm and body wall than as regards total CO_2 production. Feeding after starvation alters the condition of ectoderm and body wall but little, except gradually, as growth occurs again, consequently susceptibility of these parts is but little affected: but feeding increases greatly the metabolic activity of the alimentary tract and so increases total CO_2 production to a marked degree.

This interpretation throws no further light upon the question of the increase in capacity for acclimation following feeding after starvation. It may be, as suggested earlier (4), that this capacity is associated in some way with nutritive condition, possibly in connection with the synthesis of protein molecules under the conditions to which acclimation occurs, so that capacity for acclimation does not increase until the animal is fed after starvation.

If this interpretation is correct, the earlier conclusion that starvation brings about a rejuvenescence as regards rate of oxidation holds good, but the process may be masked, at least as regards CO_2 production, during the earlier stages by the decrease in functional activity of the alimentary tract. In some way apparently the reentrance into metabolism of substances deposited in the cells during growth and differentiation results in an acceleration of the fundamental reactions. We may interpret this change in terms of catalytic action and its products, of changes in molecular constitution, of aggregation of colloids or perhaps in other terms, but whatever these terms may be, it seems to be true that growth and differentiation are associated with a decrease, and reduction and dedifferentiation with an increase in rate of the fundamental reactions. As regards starvation, the process of rejuvenescence is not complete in all respects until the starved animal is again fed.

E. J. Lund (8) and B. L. Lund (9) have recently reported that during the starvation of Paramecium susceptibility to KNC increases while oxygen consumption decreases. On the basis of these observations they conclude that susceptibility to cyanides is simply a matter of permeability and independent of the rate of oxidation. As I have stated elsewhere (5) the susceptibility of Paramecium and various other infusoria to KNC is determined by ectoplasmic changes for it is impossible to determine with certainty when the entoplasm is affected or killed. Whether we take cessation of movement or disintegration of ectoplasm as the criterion, this is true, when the ectoplasm disintegrates the entoplasm flows out and when it dies cannot be determined with certainty. There is every reason to believe that in well-fed Paramecium, as well as in Planaria, ectoplasmic oxidation is only a small fraction of the total and that in the absence of food a marked decrease in oxidation occurs in the entoplasm. This decrease doubtless determines the decrease in total oxygen-consumption observed by Lund while conditions in the ectoplasm, which corresponds functionally to ectoderm and body wall of Planaria, bring about the increase in susceptibility. In fact the Lunds' data as far as they go are in complete agreement with my own investigations on Planaria, and as I have pointed out above, the facts indicate that the discrepancy between the data on susceptibility and those on metabolism is only apparent. Their conclusion that susceptibility to cyanide is independent of rate of oxidation is therefore not at present justified, since their own data are not complete as regards the difference in condition between ectoplasm and entoplasm, and in the light of all the facts, the opposite conclusion seems the only one possible. Lund presents no data on stages of starvation in Paramecium comparable to the late stages of starvation in Planaria, perhaps because Paramecium being a single cell, dies before such stages are attained. Nevertheless, the fact that his results on starvation obtained with a different organism and in part by a different method, agree as far as they go with my own is significant.[1] Data on susceptibility to lack of oxygen and on oxygen consumption during starvation in Planaria will be presented in later papers.

SUMMARY

1. CO_2 production decreases rapidly during the first few days of starvation in Planaria and continues to decrease more slowly during several weeks, but in advanced stages of starvation it increases.
2. The more rapidly starvation, and particularly the reduction of the alimentary tract occurs, the more rapidly these changes in CO_2 production take place. Fission and regulation accelerate the progress of starvation.
3. After three or four months of starvation CO_2 production is at least equal to or greater than that at the beginning of starvation.
4. Though the work is not yet completed, the data already obtained upon the effect of feeding after periods of starvation show that feeding after a few days of starvation raises the CO_2 production to the original level, while feeding after long periods of starvation and reduction raises the CO_2 production approximately to the level of that in young fed animals of the same size as the starved, reduced animals. In other words, the animals reduced by starvation and then fed are physiologically younger than at the beginning of starvation, and if feeding is continued are capable of undergoing growth and physiological senescence again from the stage represented at the end of the starvation period.
5. Susceptibility of the ectoderm and body wall increases from the beginning of starvation while susceptibility of the alimentary tract at first decreases very markedly and later increases. The apparent disagreement between the results of the earlier susceptibility data and the data on CO_2 production is accounted for by the great decrease in functional metabolism in the alimentary tract in the absence of food, while no such decrease occurs in body wall and ectoderm. In the earlier work susceptibility was measured in terms of ectoderm and body wall alone, but when the susceptibility of the alimentary tract is taken into account, as

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[1] Several months before the publication of his paper Lund was informed in personal correspondence of the results of my work on starvation in Planaria and the interpretation suggested by these. In this correspondence it was pointed out that the apparent discrepancy between the earlier results of the work on susceptibility and that on CO_2 production was accounted for by the fact that susceptibility was measured in terms of ectoderm and body wall, while the data on CO_2 production concerned total CO_2 production.

in later work, the results of the susceptibility method supplement and confirm the data on CO_2 production. All the facts at present known indicate that, except for the decrease in functional metabolism in the alimentary tract, the rate of oxidation increases during starvation in Planaria.

BIBLIOGRAPHY

(1) CHILD: Arch. f. Entwicklungsmech., 1911, xxxi, 537.
(2) CHILD: Journ. Exper. Zoöl., 1911, xi, 221.
(3) CHILD: Arch. f. Entwicklungsmech., 1913, xxxvii, 108.
(4) CHILD: Ibid., 1914, xxxviii, 418.
(5) CHILD: Biol. Bull., 1914, xxv, 36.
(6) CHILD: Senescence and rejuvenescence, Chicago, 1915.
(7) HAAS: Science, 1916, xliv, 1125.
(8) LUND: This Journal, 1918, xlv, 167.
(9) LUND: Biol. Bull., 1918, xxxv, 211.
(10) Tashiro: A chemical sign of life, Chicago, 1917.

THE FUNCTION OF THE NUCLEUS OF THE LIVING CELL

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METHOD

Of the different methods which have been employed to solve the problem of the function of the cell nucleus, the one which has given the most illuminating results is the method of extirpation: removing the nucleus and comparing the enucleated cell with a normal nucleated cell. In order that the nucleus may be removed from a cell or that a cell may be cut in half, it is necessary or at least expedient that a cell of considerable size be selected; but at the same time it must be a cell which can be kept alive or cultivated for several generations, if necessary. The cells which meet these requirements most exactly are certain unicellular organisms, certain protozoa. Among such a large and diverse group of organisms it is not difficult to find one which is peculiarly suited for the problem at hand. For the experiments which follow, Ameba proteus was selected. Owing to the slow movements and absence of shell in this organism, it can readily be cut in half; or if desired the nucleus may, after some practice, be removed with as little as one-tenth or less of the cytoplasm. Moreover, the lack of differentiation in Ameba is a decided advantage for we are certain that in removing part of the cytoplasm with the nucleus we are removing no important organ of the cell. The one disadvantage in the use of this organism is the difficulty of cultivating it. Like all rhizopods, amebas are difficult to raise; but many investigators have succeeded in keeping healthy cultures in the laboratory for years.

In view of the difficulties which are so often met in cultivating amebas, it may not be out of place to add one more method to those which have been described for rearing these valuable experimental animals. It is almost literally true that each investigator has a method of raising amebas which no one can use except himself. This is not surprising when it is seen that "water" is employed in making the cultures, with little regard for its precise character. While amebas may flourish in the water of one locality, they may quickly die in the water of another locality. This difficulty was avoided by the use of distilled water.[1] The method which follows is based upon one which has been employed by Miss Hyman of the University of Chicago.

A small amount of hay, including both stalks and leaves, is cut into pieces about three inches long; 4 gm. of this material are then placed in a large beaker. To this is added 0.2 gram of dry bread crumbs and 500 cc. of distilled water. The material is boiled for a few minutes and then poured into flat dishes to a depth of 1 to 2 cm. The dishes are covered to keep out dust, and if water evaporates from them it can be replaced by distilled water. After the infusion has cooled, several pipettes full of fluid are added from a culture which contains healthy amebas or from a pond or stream where amebas are found. In doing this care is taken to avoid taking up any of the sediment, for it is the sediment rather than the supernatant fluid which is apt to contain enemies to Ameba. This inoculation may be repeated. In the course of two weeks the culture becomes cloudy with bacteria, zoöglea and small ciliates and flagellates, the food of Ameba. A small dish of the culture fluid is now seeded with amebas, care being taken to avoid large organisms such as crustacea and worms. If living amebas are not present a week later, more of the fluid containing amebas should be added. This culture is used to inoculate the rest of the infusion. The addition of a little hay occasionally will keep the amebas present in great numbers. The room should not be allowed to become very hot or very cold.

The amebas were transferred to different media by means of a fine glass capillary pipette, the sharp end of which had been made smooth by passing it through a flame. In most of the experiments the animals were kept in drops of fluid upon slides with a depression at each end. The slides were kept in a moist chamber in a room whose temperature did not vary greatly from 20°C. For cutting the amebas in half or cutting the nucleus away from the rest of the cell, a fine glass needle was drawn off at a slight angle from the end of a glass rod. The cells were divided under a binocular microscope.

Since the animals would not live long in the tap water of this city, a search was made at the outset for a solution in which they could be kept in healthy, active condition. Ten amebas were removed from a culture, freed from food material and debris and transferred to the solution to be tested. Each day they were examined and transferred to a fresh solution, the experiment being continued until all of the animals were dead. It appeared at first that distilled water was the most satisfactory medium that could be obtained. Thus ten amebas lived (without food) for an average of 13.2 days in distilled water, whereas animals kept in tap water, for example, soon assumed an abnormal appearance and could seldom be kept alive longer than one week. But it was later discovered that the distilled water was greatly improved by the addition of one-tenth the volume of spring water. Not only was the average life (14½ days) somewhat longer, but the condition of the animals during this time was greatly improved. In the distilled water the animals soon cease to be attached to the bottom and are hence unable to move from place to place in the normal manner; but in the "10 per cent spring water," after it became saturated with air, they remained attached up to a few days before death. The spring water used contained a trace of calcium, but not so much as was contained in the tap water. In the following experiments the amebas, unless otherwise noted, were kept in the 10 per cent spring water.

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[1] The prejudice of many biologists against the use of distilled water and the prevalent notion that it is toxic, are not supported by experiments upon the toxicity of distilled water (Daniel, '08).

RELATION OF THE NUCLEUS TO MOVEMENT

The movement of normal amebas in a suitable medium, such as the dilute spring water, when placed upon a glass slide, was found to be of the limax type. The ameba is attached to the slide by means of some sticky secretion and flows steadily forward, sending out pseudopods at the anterior end, often from alternate sides. Frequently amebas are found which are not attached to the slide and such amebas may project pseudopods in any direction, but are unable to move from place to place. These animals will in time, however, adhere to the slide, unless they are abnormal or the medium is unsuitable. When these active amebas are stimulated roughly, as by pressure with a glass needle, they retract into a more or less spherical shape and small droplets of protoplasm may be seen projecting from the surface.

The effect of amputation of the nucleus upon the movement of the cell may be seen from the following typical experiment:

3.21 p.m. An active ameba which was attached to the slide was divided into two approximately equal parts, the nucleus remaining in the posterior half. Both parts went into the typical condition of stimulation.

3.23 p.m. Both pieces have put out pseudopods and are active, but the non-nucleated part, which was cut from the anterior end, is much more active than the nucleated and posterior part.

3.27 p.m. Both pieces are moving in the typical limax fashion, but the movement of the non-nucleated piece has become perceptibly slower.

3.28 p.m. The non-nucleated piece ceases its progressive movements and slowly retracts into a somewhat corrugated sphere. Only an occasional, very blunt pseudopod is now produced, and slight agitation shows that the fragment is no longer attached to the slide. The nucleated piece differs from a normal ameba only in size.

The retraction into the spherical shape is an invariable phenomenon and is of such a definite character that the time of its occurrence may be accurately determined. Thus in fifteen amebas which were so divided that the nucleus remained in the posterior half, the retraction occurred at an average of nine minutes after the operation. To the observer it resembles strongly the response to stimulation, except for the absence of the protoplasmic droplets mentioned above. The protoplasm is capable of movement, as is shown by transferring it to another medium such as distilled water, to which it responds by a change of shape.

This effect of amputation of the nucleus upon movement is not always permanent. If a number of amebas are cut in half and the nucleated and non-nucleated pieces observed each day, it will be noticed on the first or second day after the operation that many of the non-nucleated fragments are moving in the typical limax fashion, and in fact there is sometimes no great difference between the movements of the nucleated and the non-nucleated pieces. Slight agitation, however, shows that the non-nuclear amebas are scarcely attached to the slide; the slightest disturbance serves to dislodge them. This fact, which must be due to the failure to produce the sticky secretion, is probably partly responsible for the difference in movement between the nuclear and the non-nuclear parts.

The fact that a non-nucleated ameba may, under any conditions, exhibit normal movements, justifies us in concluding that the nucleus is not necessary for movement. In Ameba, however, movement is affected in some indirect way by removal of the nucleus.

THE SURVIVAL OF THE NON-NUCLEATED CELL

All observations made upon cells which have been deprived of their nuclei prove conclusively that non-nucleated protoplasm is destined to die without growing or dividing. But in most cases, particularly in

Ameba, it has been impossible to get this protoplasm to take in food, and even that food which is already included in the protoplasm is but imperfectly digested. Consequently it is fairer to compare the survival of an enucleated cell with one which has been isolated without food rather than with an actively feeding cell. When this is done it is found that the non-nucleated cell lives almost as long as the nucleated one, *provided there is no difference in size.* If an ameba is cut into unequal parts, the nucleus, owing to its central position, will usually be found in the larger part. Since large fragments live longer than small ones, special care must be taken to avoid a difference in size.

MEDIUM	SURVIVAL OF NUCLEATED PART	SURVIVAL OF NON-NUCLEATED PART
	days	*days*
Distilled water	7	6
Distilled water	7	7
Distilled water	10	6
Distilled water	9	4
Distilled water	7	4
Distilled water	5	3
Distilled water	9	6
Culture water	7	5
Culture water	8	6
Culture water	6	6
Culture water	9	7
Culture water	11	8
Culture water	7	8
Culture water	7	7
Culture water	7	7
Average	7.7	6

Fifteen amebas were cut in half and both halves were kept without food in either distilled water or in water taken from the culture. The number of days which each piece lived after the operation is given in the above table.

It will be noted that in some cases the non-nucleated fragments lived as long as those which contained nuclei. In fact by cutting the ameba in such a way that the piece containing the nucleus was smaller than the piece which lacked it, the non-nucleated piece could be made to outlive the nucleated one. Thus ten amebas were divided in such a way that the nucleated piece was from one-half to one-fourth the size of the other piece. The results are tabulated below:

	SURVIVAL OF NUCLEATED PART	SURVIVAL OF NON-NUCLEATED PART
	days	*days*
	6	10
	7	7
	3	13
	6	5
	3	6
	6	7
	7	7
	6	8
	7	7
	6	10
Average	5.7	8

We may conclude that a cell deprived of its nucleus may survive as long as a cell deprived of food.

THE NUTRITION OF AMEBA; THE USE OF SUBSTANCES IN SOLUTION

The apparent resemblance of enucleated amebas to amebas deprived of food suggested that some of the phenomena exhibited by the non-nucleated cell might be the result of starvation and have no direct connection with the absence of a nucleus. Accordingly, an attempt was made to supply the amebas with adequate food substances. Since it was not possible to get the enucleated organisms to take in food particles, an effort was made to provide a medium in which amebas could be nourished by the absorption of substances in solution.

It has been pointed out (Burrows and Neymann, '17) that no artificial synthetic medium has ever been provided which was adequate to nourish an animal cell although the blood and lymph apparently constitute a natural synthetic medium for the cells of higher organisms. Even cells cultivated in vitro depend upon the autolysis of neighboring cells for their nourishment, and the attempt to cultivate protozoa in nutritive organic media has met with complete failure (Biedermann, '16; Doflein, '11). In the light of recent experiments in nutrition, however, it seems possible that such negative results may be due to the failure to supply certain substances which are necessary for proper nutrition. An attempt was therefore made to provide an adequate medium for the primitive animal cell, Ameba proteus.

Since amebas were found to move more normally and to live longer in distilled water to which had been added one-tenth the volume of spring water, this "10 per cent spring water" was used in making all solutions. Various carbon compounds, including several sugars, were now tested with the hope of finding a source of energy.

DATE	NUMBER OF AMEBAS LIVING		
	Water	1 per cent glucose	1 per cent levulose
February 7	20	20	20
February 8	20	20	20
February 9	18	20	20
February 10	15	20	20
February 11	15	20	20
February 12	15	20	20
February 13	13	20	20
February 14	13	20	20
February 15	13	20	20
February 16	13	20	19
February 17	13	20	19
February 18	13	20	18
February 19	13	20	18
February 20	11	18	17
February 21	11	17	17
February 22	11	17	15
February 23	9	17	15
February 24	7	17	15
February 25	6	16	14
February 26	6	16	12
February 27	4	12	11
February 28	3	12	8
March 1	2	11	8
March 2	2	10	2
March 3	2	6	0
March 4	1	4	
March 5	1	3	
March 6	1	0	
March 7	1		
March 8	0		
Average life	13.6 days	21.8 days	19.4 days

The method used was as follows: A number of amebas were selected from the same culture and freed from food and bacteria by transferring repeatedly to fresh solutions of 10 per cent spring water. They were then transferred to slides, placing ten in 10 per cent spring water as a control, and ten in each of the solutions to be tested. The animals were examined and counted each day, washed in water and transferred to fresh solutions to prevent the development of bacteria. The most promising substances found were the hexoses, glucose and levulose, the effect of which may be seen in the preceding experiment.

DATE	NUMBER OF AMEBAS LIVING		
	1 per cent glucose	1 per cent glucose + 0.1 per cent urea	1 per cent glucose + 0.01 per cent NH_4NO_3
February 24	10	10	10
February 25	9	10	8
February 26	9	11*	8
February 27	9	12	8
February 28	6	12	8
March 1	5	12	8
March 2	5	13	8
March 3	4	12	8
March 4	4	12	8
March 5	4	12	8
March 6	4	10	8
March 7	4	10	8
March 8	4	10	8
March 9	4	8	7
March 10	4	6	7
March 11	4	6	7
March 12	4	6	4
March 13	3	5	3
March 14	2	4	2
March 15	1	3	2
March 16	1	3	1
March 17	1	3	0
March 21	0	1	
March 22		1	
March 27		0	
Average life	10.4 days	18.9 days	13.9 days

* The increase in number shown in this and subsequent tables is the result of the division of certain of the amebas.

Not only did the amebas live longer in the sugar solutions but during the early part of the experiment they were much more active. At the end of a week, however, many of the amebas began to take on an opaque appearance and ceased normal movements. The substance which was formerly a food now acted as a poison. Child ('15) has shown that susceptibility to poisons increases during starvation.

Although these cultures were not sterile, it was possible to keep the bacteria from becoming numerous and thus prevent their becoming an appreciable source of food for the amebas.

Having found a carbohydrate food for Ameba, a search was now made for a source of nitrogen. In such a primitive cell the possibility that simple compounds of nitrogen may be used at once suggests itself. Of various compounds of nitrogen which were tried, the best results were obtained with ammonium nitrate and urea; and of these two, urea was much the better owing to its comparatively low toxicity. The effect of these substances is seen in the preceding experiment. (The comparatively short life of the animals is to be attributed to the use of an inferior culture.)

That these compounds are of some use to the amebas is indicated by the increased length of life when they are added to the solution; but that they are not an adequate source of nitrogen is indicated by the fact that the animals eventually die and that no growth can be detected. The shrinkage caused by the glucose would render the detection of growth difficult even if present.

Of special interest, however, are the three cases of cell division in the urea solution. Although hundreds of amebas have been kept under daily observation until they were completely disintegrated, no single case of cell division was ever observed unless food substances were supplied; and with two possible exceptions, no division was observed unless a source of nitrogen was added. On the other hand, divisions have been repeatedly observed in solutions containing urea or certain amino acids. That the division is not simply the result of the stimulating action of the urea upon the protoplasm is shown by the effect of urea solutions to which no glucose is added. In such solutions the amebas not only do not divide, but they die more quickly than in water alone. Urea in the absence of glucose is simply a mild poison but when urea and glucose are used together, the animals live longer, have a more normal appearance and may even reproduce. The necessity of using the two substances together indicates that they are built up or combined to form some more complex substance which is of use to the organism.

If this is a true case of organic synthesis, it would be interesting to see whether it occurs in the cell which has been deprived of its nucleus, in order to throw light upon the supposed synthetic function of the nucleus. This point is taken up later.

Having failed to obtain growth with simple compounds of nitrogen, the organisms were now supplied with amino acids. The use of simple amino acids was not promising. Some reproduction was obtained with a saturated solution of tyrosin, but the animals soon died. A mixture of amino acids was then prepared in the following way:

Five grams of Hammarsten's casein was heated for twenty hours with 100 cc. of a molecular solution of sulphuric acid. The hydrolysis was performed on a water bath in a flask fitted with a reflux condenser. At the end of the hydrolysis, when the solution no longer gave the biuret reaction, a saturated solution of barium hydroxide was added until the reaction was slightly alkaline, and the solution boiled to remove ammonia. Dilute sulphuric acid was added until the reaction was very slightly acid, and the solution filtered hot. The barium sulphate precipitate, which had absorbed much of the humus substance, was not washed. The solution was exactly neutralized with sodium hydroxide, and the resulting fluid, whose volume was 700 cc., was clear and straw-colored and had a bitter-sweet taste. The reactions for barium, calcium, iron, reduced sulphur and tryptophane were negative; phosphate was present and the xantho-proteic reaction was positive. When exposed to the air, bacteria developed with remarkable rapidity. The solution was not toxic to amebas when evaporated to one-half the volume, but evaporation to one-fifth the volume resulted in a solution which was slightly toxic.[2]

This solution of amino acids lacks glycine, which is absent in casein, and tryptophane and cystine, which were destroyed.

In cultivating amebas in this solution it was difficult to prevent the development of bacteria, but a method was devised which served to exclude them almost completely. The amebas were washed five to ten times in sterile water, and the slides upon which they were kept were boiled. The culture solutions were kept in small flasks fitted with capillary tubes. By inverting the flask and warming with the hand, a few drops could be placed upon the slide. After using, the flask was boiled and a test tube placed over its neck. The cultures were examined daily, the amebas washed in sterile water and transferred to new solutions.

The hydrolyzed casein solution proved to be a fairly good medium in which cases of cell division were occasionally observed, but there was never any growth and the changes which preceded death could be seen in about ten days. The addition of glucose resulted in some improvement but it did not prevent death.

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[2] The toxicity of amino acids observed by Burrows and Neymann ('17) upon embryonic chicken cells was probably due to the relatively concentrated solutions used.

DATE	NUMBER OF AMEBAS LIVING	
	Hydrolyzed casein	Hydrolyzed casein + 0.2 per cent glucose
May 1	5	5
May 2	5	6*
May 3	6*	6
May 4	6	6
May 5	6	7
May 6	6	8
May 7	6	8
May 8	Dying	8
May 9	Discontinued	8
May 10		8
May 11		8
May 12		8 Amebas healthy and active
May 13		8 Amebas somewhat abnormal
May 14		7
		Dying. Experiment discontinued

* See footnote to table, p. 265.

DATE	NUMBER OF AMEBAS LIVING
	Hydrolyzed casein + 1 per cent glucose + 0.1 per cent tryptophane + 0.08 per cent cystine + 0.2 per cent milk.
May 23	5
May 24	5
May 25	6*
May 26	6
May 27	6
May 28	6
May 29	6
May 30	6
May 31	6
June 1	6
June 2	6
June 3	5
June 4	3
June 5	3
June 6	1
June 7	0

* See footnote to table p. 265.

Since this death might be due to the absence of some foodstuff, various substances were added to the "diet." The addition of various salts was tried but no improvement was observed. Cystine and tryptophane, so important in the nutrition of higher animals, were added to the solution in concentrations which were shown not to be toxic. A small amount of milk was added to provide the unknown "accessory" food substances, and also certain salts (see lower table on page 268).

No synthetic medium was found for Ameba proteus. The failure may be due to some peculiarity of the cell, such as its enormous size, or to a low permeability.

THE NUTRITION OF THE NON-NUCLEATED CELL

Since, as was shown above, glucose prolongs the life of amebas and apparently acts as a food, it would be interesting to know whether it has a similar effect upon the cell from which the nucleus has been removed. To determine this, the nucleus was removed from a number of amebas, care being taken to remove as little cytoplasm as possible. Under favorable circumstances it is possible to cut the nucleus from the cell without removing more than one-tenth of the cytoplasm. Some of the enucleated amebas were now transferred to water while an equal number of others of equal size were kept in glucose solutions. The usual precautions were taken to prevent the development of bacteria.

DATE	NUMBER OF AMEBAS LIVING	
	Water	0.5 per cent glucose
December 3	9	9
December 4	9	9
December 5	9	9
December 6	9	9
December 7	9	9
December 8	9	9
December 9	9	9
December 10	9	9
December 11	8	8
December 12	4	7
December 13	3	6
December 14	2	4
December 15	0	3
December 16		1
December 17		0
Average life	9.9 days	11.2 days

Although the amebas kept in glucose lived somewhat longer than the controls, the effect was not very striking. Since there are great differences between different amebas, even when selected from the same culture, a somewhat more satisfactory experiment was performed as follows: The nucleus was removed from a number of amebas and then the amebas were divided in half. One-half was placed in water, as a control, while the other half was kept in a solution of glucose.

DATE	NUMBER OF AMEBAS LIVING	
	Water	1 per cent glucose
April 3	12	12
April 4	12	12
April 5	12	12
April 6	12	12
April 7	11	11
April 8	10	11
April 9	9	11
April 10	6	10
April 11	3	6
April 12	2	4
April 13	0	3
April 14		0
Average life	7.4 days	8.7 days

DATE	NUMBER OF AMEBAS LIVING		
	Glucose	Glucose + 0.1 per cent urea	Glucose + 0.01 per cent NH_4NO_3
November 19	10	10	9
November 20	10	10	9
November 21	10	10	9
November 22	10	9	9
November 23	10	9	8
November 24	10	7	8
November 25	10	5	8
November 26	9	2	7
November 27	6	1	5
November 28	5	0	2
November 29	4		2
December 2	0		1
December 3			0
Average life	10.2 days	6.3 days	9.0 days

The non-nucleated cells live longer in glucose solutions and probably use it as a food. This is in agreement with the work of Rous and Turner ('15), who used glucose for the preservation of the non-nucleated red blood corpuscles.

If the life of the non-nucleated cell is prolonged by the use of glucose, is it further prolonged by the use of urea or ammonium nitrate?

The nucleus was removed from twenty-nine amebas, and after washing, ten were placed in glucose, ten in glucose plus urea, and nine in glucose plus ammonium nitrate (see lower table on page 270).

Thus in the non-nucleated cell, the addition of urea to the glucose was not beneficial; on the contrary it was harmful. The addition of the ammonium nitrate was without effect.

Evidence was offered above that the normal, nucleated ameba was able to form some combination between glucose and urea (or some derivative of urea). If it is true that the beneficial effect of glucose plus urea depends upon a synthesis, we may conclude that the non-nucleated cell is unable to perform this synthesis.

RESPIRATION IN THE NON-NUCLEATED CELL

It has been suggested (Loeb, '05) that the nucleus is the organ of oxidation of the living cell and that peculiarities of the non-nucleated cell, such as lack of the power to synthesize or to regenerate lost parts, are the result of the lowered oxidative activity. The non-nucleated pieces, it is said, "die slowly from asphyxia."

If this theory is true, we should expect to find a marked difference between the effect of depriving the non-nucleated cell of oxygen and the effect of depriving the nucleated cell of oxygen. We should expect the cell in which oxidations were occurring most rapidly to be most affected by its removal, whereas the cell which was using very little oxygen should not be greatly affected. According to Child ('15), regions in which respiration is rapid are more susceptible to lack of oxygen and die sooner than regions in which respiration is slower.

The effect of depriving the nucleated and non-nucleated cells of oxygen was investigated in the following manner: Five or more amebas were divided into two pieces of as nearly equal size as possible. After leaving them for a longer or shorter period in air, they were placed in hanging drops, side by side, in an Engelmann gas-chamber. Nitrogen, which had been washed in water, was then passed through the chamber, and this atmosphere was maintained until the death of the organisms.

In the third experiment the nitrogen was first bubbled from a capillary tube through strongly alkaline pyrogallic acid, to remove possible traces of oxygen. In the first experiment, the temperature of the water jacket surrounding the chamber was raised to 26°C. in order to accelerate the experiment. The other experiments were performed at 20°C.

Experiment 1

TIME	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
5.00 p.m.	Amebas divided	
9.50 a.m.	Placed in nitrogen	
10.00 a.m.	7	7
11.00 a.m.	7	7
12.00 a.m.	7	6
1.00 p.m.	7	4
1.20 p.m.	7	3
1.30 p.m.	6	1
2.00 p.m.	4	0
2.25 p.m.	3	
2.30 p.m.	2	
2.35 p.m.	1	
2.45 p.m.	0	
Average life	4.4 hours	3.4 hours

Thus there is a distinct difference in the susceptibility to lack of oxygen in the nucleated and non-nucleated cell, but this difference is just the opposite to what we should expect if oxidations were proceeding more rapidly in the nucleated half. Not only does the non-nucleated cell die more quickly when deprived of oxygen, but it is the first to assume a spherical shape and to cease putting out pseudopods.

The opposite experiment, that of increasing the supply of oxygen, is also of interest. Were it true that in removing the nucleus we have removed the organ of oxidation, and that the cell is slowly dying of asphyxia, it should be possible to delay the death of the non-nucleated cell by supplying it with more oxygen. On the contrary, it was found that when the nucleated and non-nucleated halves of amebas were kept in an atmosphere of oxygen, both died in less than twelve hours and the non-nucleated pieces were killed quite as rapidly as those which contained nuclei.

Experiment 2

TIME	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
10.30 a.m.	Amebas divided	
11.05 a.m.	Placed in nitrogen	
12.00 a.m.	5	5
1.00 p.m.	5	5
2.00 p.m.	5	5
3.00 p.m.	5	5
4.00 p.m.	5	5
5.00 p.m.	5	5
6.00 p.m.	5	5
7.00 p.m.	5	4
7.30 p.m.	5	2
8.30 p.m.	4	2
9.00 p.m.	4	2
10.00 p.m.	4	1
11.00 p.m.	4	1
12.00 p.m.	4	1
8.00 a.m.	3	1
9.00 a.m.	3	1
9.30 a.m.	3	0
10.00 a.m.	3	
10.15 a.m.	1	
11.00 a.m.	1	
12.00 a.m.	1	
1.00 p.m.	1	
2.00 p.m.	0	
Average life	20.5 hours	11.6 hours

Experiment 3

TIME	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
9.00 a.m.	Amebas divided	
10.20 a.m.	Placed in nitrogen	
11.00 a.m.	5	5
12.00 a.m.	5	5
1.00 p.m.	5	5
3.00 p.m.	5	4
4.00 p.m.	5	2
5.00 p.m.	5	2
8.00 p.m.	5	1
9.00 p.m.	4	1
9.30 p.m.	4	0
10.00 p.m.	2	
11.00 p.m.	2	
12.00 p.m.	2	
6.00 a.m.	0	
Average life	14.6 hours	7.4 hours

The experiment was performed in the following manner: Five amebas were cut in half with a fine glass needle, and each half was transferred to a hanging drop in a gas chamber. Oxygen which had been thoroughly washed was now passed through the chamber and the organisms kept under observation until all had disintegrated. The animals were divided at 10 a.m. Oxygen was passed through at 10.30 a.m.

Time of death

NUCLEATED CELL	NON-NUCLEATED CELL
1.30	2.00
2.00	2.10
4.15	3.23
5.00	3.27
6.30	4.15
Average life... 5.4 hours	4.6 hours

The injurious action of oxygen, which is doubtless the result of an increase in the oxidations of the cell, took effect somewhat more rapidly upon the cell which lacked a nucleus. It is difficult to see how a cell could be killed in a few hours by an atmosphere of oxygen if its oxidations have become depressed.

THE EFFECT OF TEMPERATURE UPON THE NON-NUCLEATED CELL

In the experiments hitherto reported, a fairly constant temperature of 20°C. had been maintained. An attempt was now made to determine the effect of temperature upon the nucleated and non-nucleated cells, with the hope of discovering a possible difference in the rate at which chemical changes were occurring in the two cells. Two methods of investigation suggest themselves:

1. The temperature of the medium may be gradually raised until death occurs.
2. The organisms may be kept at a rather high temperature and observed until death occurs.

Both of these methods were used in the order named.

In the first method amebas were placed upon a Pfeiffer warming stage, through which water of any desired temperature could be passed. After dividing the ameba into two equal parts, the temperature of the warming stage was raised at the rate of about one degree Centigrade per minute. The rate at which the temperature is raised is of importance for by raising the temperature rapidly the organisms may be killed at a comparatively low temperature. As the temperature rises, the amebas soon withdraw all of their pseudopods and assume a spherical shape. This does not, however, indicate the death of the organism for if at this point the temperature is slowly lowered to 20°C. the animals will, in a few hours, send out pseudopods and resume normal movement. If the temperature is raised high enough, the cells will either disintegrate completely or coagulate, with the result that the cell boundary can no longer be seen. The temperature at which the spherical shape was assumed and the temperature at which death occurred are recorded below.

EXPERIMENT	SPHERICAL SHAPE ASSUMED		DEATH	
	Nucleated	Non-nucleated	Nucleated	Non-nucleated
	°C.	°C.	°C.	°C.
1	39	38	51	50
2	40	39	50	50+
3	40	40	47	52
4	39	44	49	48
Average.......	39.5	40.2	49.2	50.1

The effect of gradual cooling was not so easily determined. When the temperature of the fluid was lowered greatly and even under-cooled, there was no injury provided crystallization did not take place. When the solution crystallized, however, as the result of adding a small crystal of ice, the amebas were injured mechanically and disintegrated as soon as the crystals melted.

But the effect of cooling could be shown in a somewhat different manner. The nucleated and non-nucleated parts of an ameba were slowly warmed to 44°C. and then cooled in ten minutes to 22°C. Two minutes later both fragments disintegrated at the same time, the result of the rapid cooling.

Thus no difference could be found in the susceptibility of the nuclear and non-nuclear fragments to a rise or fall of temperature. The other method was now employed: keeping amebas at different temperatures until death occurred.

Eleven amebas were freed from food particles and debris and divided into two equal parts. Both the nuclear and non-nuclear fragments were kept in a moist chamber at an average room temperature of 20°C.

Experiment 1. 20°C.

DATE	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
October 10	11	11
October 11	11	11
October 12	11	11
October 13	8	10
October 14	7	6
October 15, a.m.	7	3
October 15, p.m.	5	2
October 16	4	2
October 17	4	2
October 18	4	2
October 19, a.m.	3	1
October 19, p.m.	1	0
October 20	0	
Average life	6.2 days	5.3 days

Thus, as was shown before, the life of the non-nuclear fragment at ordinary temperature is almost as long as that of the nuclear fragment.

Ten amebas were now divided and each placed in a moist chamber which was kept in a thermostat at 30°C.

Experiment 2. 30°C.

DATE	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
October 22	10	10
October 23, a.m.	10	7
October 23, p.m.	9	7
October 24	9	4
October 25	9	2
October 26, a.m.	6	1
October 26, p.m.	5	0
October 27	3	
October 29	3	
October 30	3	
October 31	1	
November 1	0	
Average life	6.0 days	2.4 days

Apparently the life of the nuclear fragments was not shortened by exposure to high temperature, but this was probably due to the use of healthier amebas in experiment 2. Further experiments showed that the life of nucleated amebas was shortened by raising the temperature but the life of non-nuclear amebas was shortened somewhat more.

Twelve amebas were now divided and placed in a moist chamber at 10°C. The moist chamber was allowed to warm slowly to room temperature before removing the slide for examination.

Experiment 3. 10°C.

DATE	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
October 17	12	12
October 18	12	12
October 19	12	12
October 20	12	12
October 21	12	12
October 22	12	12
October 23	11	11
October 24	11	11
October 25	11	11
October 26	11	11
October 27	11	11
October 29, a.m.	11	9
October 29, p.m.	11	8
October 30, a.m.	11	5
October 30, p.m.	11	4
October 31, a.m.	11	3
October 31, p.m.	10	2
November 1, a.m.	5	0
November 1, p.m.	3	
November 2	0	
Average life	14.5 days	12.8 days

The life of both fragments was prolonged by cold in this case but in two other cases it was distinctly shortened. This may have been due to the use of different cultures.

If susceptibility to high or low temperature may be used as an index to the rate of metabolism, there is no very great difference between the two fragments. There is some evidence that cells in which metabolic processes are rapid show a greater susceptibility to high temperature than do cells with a low metabolism (Child, '15, p. 66). If this is true, there is some slight indication of a more rapid metabolism in the non-nuclear fragment.

METABOLIC RATE IN THE NON-NUCLEATED CELL

The rate at which metabolic processes are occurring in different parts of an organism or tissue has been very successfully studied by determining their susceptibility to various poisons, especially to potassium cyanide (Child, '13, '15). Child has brought together a great deal of direct and indirect evidence to show that, in general, regions of high metabolism are more susceptible to poisons than are regions of low metabolism. Accordingly, the susceptibility of the nucleated and non-nucleated cell was investigated with a view to determining how the rate of metabolism was affected by the removal of the nucleus.

The amebas were cut in half and one hour after the operation they were transferred to a fresh $\frac{M}{50}$ solution of KCN upon a hollow-ground glass slide. The drop of solution was immediately covered with a cover slip to prevent the escape of HCN. Since this could not be entirely prevented, the solution gradually became weaker and as a result those amebas which were not killed in two or three minutes usually lived for more than an hour. Since small fragments of Ameba were found to be more susceptible than large ones, an effort was made to divide the amebas into equal parts. After more than fifty Amebas had been treated with cyanide, it was obvious that some unknown factor was playing a part in the death of the organisms; for although the non-nucleated fragments usually succumbed first, there were many conspicuous cases in which the nucleated part died much sooner. It seemed possible that the unknown factor might be the difference between the anterior and posterior end. The amebas were divided when they were moving along the bottom in the "limax" condition, and in some cases the non-nuclear fragment was taken from the flowing anterior end while in other cases it was taken from the retracting posterior end. In order to determine whether this made a difference, a number of amebas were divided in such a way that the nucleus remained in the anterior half, while others were so divided that the nucleus remained in the posterior half.

The number of minutes which the nuclear and non-nuclear fragments were able to withstand treatment with cyanide is tabulated below:

Duration of life

NUCLEUS IN ANTERIOR END		NUCLEUS IN POSTERIOR END	
Nucleated	Non-nucleated	Nucleated	Non-nucleated
min.	*min.*	*min.*	*min.*
96	8	190	83
5	34	155	10
45	15	145	5
200	200	85	1
190	190	170	167
45	170	5	5
260	240	145	115
1	2	146	2
290	530	105	2
580	62	100	1
6	180	98	7
2	140	5	3
260	1	1.5	1
260	25	1.9	0.8
300	15	76	2
230	3	74	2
230	140	6	2
320	305	80	11
87	2	80	10
230	6	75	5
185	41	1	0.5
255	450	12	1
450	250	9	3
2	445	25	25
4	100		
305	1		
10	1		
120	1		
1	0.5		
410	410		
330	330		
320	1		
Average: 188.4	134.3	74.6	19.3

When the nucleus was in the anterior end, the non-nuclear fragment died first in 19 cases, the nuclear fragment died first in 7 cases and in the remaining 4 cases each part disintegrated at about the same time. When the nucleus was in the posterior end, however, the nuclear fragment was never the first to disintegrate; the death of both fragments occurred at the same time in two instances, and in the remaining 22 cases the non-nuclear fragment was the first to disintegate. The complicating factor is now understood. The anterior end of Ameba is more susceptible to cyanide than the posterior end. Essentially the same conclusion was reached by Miss Hyman ('17) in an investigation of the metabolic gradient in Ameba.

When the non-nucleated half is taken from the posterior end, its greater susceptibility to cyanide is not very definite because of the difference which exists between the anterior and posterior parts. When this disturbing factor is removed, however, by cutting the non-nuclear fragment from the anterior end, the non-nuclear fragment is seen to be much more susceptible to cyanide than the nuclear fragment.

The same difference in susceptibility between the nucleated and non-nucleated cells was found when they were kept for twenty hours after the operation before treatment with cyanide.

Whether we can conclude that the rate of metabolism is increased by removal of the nucleus is doubtful. It is possible that removal of the nucleus decreases the resistance to cyanides for some other reason. But, at any rate, the evidence such as it is opposes the view that respiration and metabolism are depressed by removal of the nucleus.

SUMMARY AND CONCLUSIONS

1. An ameba from which the nucleus has been removed may at times exhibit perfectly normal movement; in general, however, movement is somewhat affected by removal of the nucleus.
2. An ameba deprived of its nucleus lives almost as long as an ameba deprived of food.
3. Evidence is offered that Ameba can use glucose in solution as a food. There is also evidence that Ameba can synthesize glucose and urea, or some derivatives of these substances, to form a product which is of nutritive value.
4. Glucose is also of some benefit to the enucleated ameba but the supposed synthesis of glucose and urea can not be performed.
5. The non-nucleated cell is injured more quickly by either a lack or an excess of oxygen than is the normal nucleated cell.
6. The non-nucleated cell is somewhat more susceptible to high or low temperature than the nucleated cell.
7. The non-nucleated cell is more susceptible to cyanide than the nucleated cell.

DISCUSSION

There are two important theories of nuclear function: the theory of *synthesis* and the theory of *oxidation*. According to the former theory, the non-nucleated cell is unable to construct or synthesize new substances; while according to the latter, it is more or less unable to oxidize the substances already present. In reviewing the experiments here reported it is seen that they not only offer strong evidence for the validity of the synthesis theory, but they constitute a practical proof that the oxidation theory is not correct.

From the outset there has been no satisfactory evidence in support of the oxidation theory. It was apparently suggested by some experiments reported by Spitzer ('97). Spitzer obtained from various cells a preparation of nucleoprotein which contained a small amount of iron and which had the property of decomposing hydrogen peroxide. From this it has been concluded not only that all nuclei contain iron, but even that all nucleoproteins contain iron. The latter statement is of course false, and the microchemical evidence in support of the former statement (Macallum, '92) is very unsatisfactory.[3] The presence of iron in the nucleus was taken as an indication that the nucleus played an important rôle in oxidations, but the presence of this iron is open to doubt. Spitzer's catalase may have been derived from the cytoplasm.

The chemical evidence for the oxidation theory is very slight and the physiological evidence is equally so. The assumption that the organ which is furthest removed from the supply of oxygen is the organ of oxidation is not probable in the first place. "It should be borne in mind that oxygen, in order to reach the nucleus, must penetrate a layer of cytoplasm containing reducing substances." (R. S. Lillie, '11.) Moreover, as was shown in the above experiments, the non-nucleated cell may exhibit perfectly normal movements. Since the energy for movement in aerobic organisms is derived from the oxidation of organic matter, it is unlikely that oxidations have become depressed. But if there is a depression of oxidations we should certainly expect (cf. Loeb, '05) to find some improvement in the cell when we increase its supply of oxygen. On the contrary, as has been shown, the cell becomes spherical and dies in a few hours. Also the susceptibility to lack of oxygen, the susceptibility to cyanide and the susceptibility to high and low temperature all indicate that respiration and rate of metabolism are quite as rapid in the enucleated cell as in the normal cell.

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[3] From a very pure preparation of the sperm heads of the Great Lakes Whitefish, the author was unable to find a trace of iron in 0.5 gram of the dry material by the sulphocyanide test. The ashing was done in the electric muffle and by Neumann digestion, so that there was no question of "masked" iron. (Unpublished.)

Recently, however, experiments have been reported which seem to give strong support to this theory; namely, the experiments of Osterhout on the leaf of the Indian Pipe. When the leaf of this plant is cut, the cells which have been injured soon become dark. Osterhout ('17) has shown that this darkening is an oxidation and since it occurs first in the nucleus, he argues that the nucleus is the center of oxidations. These experiments, however, admit of another interpretation. It is reasonable to believe that at the center of the cell there is a strong reducing action and that very little oxygen is present. After the death of the cell, however, oxygen, like other substances, may enter freely, and in the *dying* cell it would be expected that oxidations would be very rapid in the neighborhood of the nucleus, where the reducing action is greatest.

Moreover, Kite and Chambers ('12) found, in cells which were apparently uninjured, that *reduction* was most rapid in the region of the nucleus. The dye janus green becomes red when reduced. Kite and Chambers observed that this reduction occurred first at the centrosome and second in the chromatin of the nucleus.

The experiments here reported offer support to the theory that the nucleus is the organ of synthesis. When a cell is deprived of food the processes of synthesis must, to a great extent, come to a stop, although synthesis of intermediary products of metabolism is still possible. It is probably not a coincidence that a cell deprived of its nucleus lives almost as long as a cell deprived of food.

An opportunity to put the synthesis theory to a direct test was afforded by nutritional experiments. When amebas are kept without food they will die in from one to two weeks, but if glucose is present in solution their life is somewhat prolonged. It appears that the glucose is used as a food. When urea was added the organisms lived even longer and in some cases divided. Urea alone, without glucose, had no such effect, a fact which is interpreted as meaning that the nitrogen compound is combined by the cell with the carbon compound. When nitrogen was already combined with unoxidized carbon, as in the amino-acids, the addition of glucose was unnecessary.

When these experiments were repeated upon the enucleated cell, it was found that glucose was somewhat beneficial and was apparently used as a food. But the further addition of urea was not only of no benefit, but actually hastened the death of the protoplasm. Apparently non-nuclear protoplasm is unable to combine carbon and nitrogen: the power of synthesis is absent.[4]

There is further evidence of the loss of the power of synthesis in the failure to produce the sticky secretion by means of which Ameba attaches itself to various objects. Normal amebas are firmly attached to the slide and frequently stick to the sides of a pipette when drawn up into it. Non-nucleated amebas, however, shortly after the removal of the nucleus, are but slightly if at all attached to the slide and never stick to the sides of a pipette.

The enucleated cell may move, respire, digest, respond to stimuli and exhibit any activity which is dependent solely upon catabolic or destructive processes of protoplasm. The group of phenomena which it never shows are those of growth and of regeneration and division. The phenomena of growth are essentially phenomena of organic synthesis, and the dependence of growth upon the nucleus implies the dependence of organic synthesis upon the nucleus.

BIBLIOGRAPHY

Biedermann '16. Winterstein's Handb., xxi, 278.
Burrows and Neymann. '17. Journ. Exper. Med., xxv, 93.
Child. '13. Journ. Exper. Zoöl., xiv, 153.
'15. Senescence and rejuvenescence, Chicago.
Daniel. '08. This Journal, xxiii, 48.
Doflein. '11. Lehrbuch der Protozoenkunde, 298.
Hyman. '17. Journ. Exper. Zoöl., xxiv, 55.
Kite and Chambers. '12. Science, N.S., xxxvi, 639.
Lillie. '11. Journ. Morphol., xxii, 722.
Loeb. '05. Studies in general physiology, ii, 505.
Macallum. '92. Proc. Royal Soc., London, 1.
Osterhout. '17. Science, N.S., lvi, 367.
Rous and Turner. '15. Proc. Soc. Exper. Biol. and Med., xii, 122.
Spitzer. '79. Pflüger's Arch., lxvii, 615.

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[4] The configuration of atoms: C-N-C, so characteristic of the nucleic acid molecule, is also the configuration which occurs in the protein molecule at the points where the amino acids are joined. This fact may be not without significance.

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A PLETHYSMOGRAPHIC STUDY OF SHOCK AND STAMMERING

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The experimental work here reported was begun in May, 1917, and continued until October, 1918. The intention at the start was to test the organic reactions accompanying stammering, with special reference to Dr. C. S. Bluemel's cerebral congestion theory of stammering, and this object has been kept in mind throughout.

As I was unable to secure as subjects any stammerers who had been trephined and as it has been shown that the changes in volume in the hand or finger represented the changes in all parts of the periphery, the only expressive process studied was the change in volume of either the right hand or the second finger of the right hand.

The introspections of all severe stammerers agree that they frequently experience, when called upon to say something of importance, a great fear that they will stammer if they attempt to speak. This fear sometimes is so intense that the stammerer's mind becomes a blank and he stands immovable and speechless; his eyes bulge from their sockets, the perspiration streams down his back, he opens his mouth and may even tremble, thus exhibiting all the symptoms of real fright. It seemed worth while, therefore, to arouse, in both normal speakers and stammerers, an emotion similar to that of fright by producing shock by such means as the unexpected blast of a shrill whistle, and to compare the reactions of normal speakers to shock with those of stammerers to shock, and the stammerer's reactions to shock with his reactions to stammering.[1]

Before describing my experiments, it will be well to review briefly the results obtained by other investigators.

History of experimental literature. It is needless to review in detail the investigations included by Anderson (1), Angell and Thompson (3), Shepard (29), Stevens (32) or Weber (35) in their historical résumés. I will begin, therefore, with a summary of the results obtained by all the experimenters who have, to my knowledge, worked on this problem, and I will then review the results obtained by two recent writers whose work has a special bearing on my experiment.

Table 1 gives the direction of the changes in arm, brain and intestinal volume during fright, sensory stimulation, pleasant versus unpleasant emotions or sensations, tension versus relaxation, physical work and mental work, as reported by every experimenter who has to my knowledge worked on this problem, together with the dates when the experiments were performed if they are given (otherwise the date of the publication), the number of subjects used by each experimenter (where given), and the number of experiments performed by each experimenter (where given).

All five experimenters who obtained reactions to fright found that fright almost always occasioned a marked decrease in peripheral volume, with or without a temporary rise when stimulus was given; Küppers alone reported an increase in a small percentage of his experiments. Weber found an increase in intestinal volume. The three who experimented on the brain found an increase in brain volume at some point on the record; Berger found an increase followed by a decrease, Mosso an increase only, Shepard a short increase followed by a brief return to normal, then a long characteristic increase.

Fourteen experimenters found that sensory stimuli, in a very large majority of cases, brought about a decrease in arm volume less marked than in fright, and preceded less often by a temporary rise; Lehmann alone reported an increase for touch stimuli and a decrease both preceded and followed by a temporary increase for visual and acoustic stimuli. All three experimenters found a rise in brain volume.

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[1] It is unfortunate that some American writers have employed misleading definitions for "stammer" and "stutter." Some writers use "stammer" to denote a stoppage of speech and "stutter" to denote the repetition of the first letter or syllable of certain words. Shepard and some other writers use "stutter" to denote both repetition and stoppage, and "stammer" to denote mispronunciation, as in baby talk and lisping. As stuttering usually develops into stammering and as many defects of speech consist of both as first defined, I shall use "stammer" throughout this discussion as absolutely synonymous with "stutter" as defined in Webster's *New International Dictionary*—"to make involuntary stops in uttering syllables or words."

Sixteen experimenters compared the vasomotor changes in the periphery accompanying pleasant versus unpleasant emotions and sensations, and all found that the unpleasant states brought about constriction. Eleven found that the vasomotor changes were the same for pleasant as for unpleasant states; five found the opposite to be the case. As but five experimenters, whose interpretations of their results are questionable, found opposite vasomotor changes for pleasant and unpleasant states in but few experiments on but few subjects, and as eleven experimenters, including the most accurate experimenters we have, found no difference in the reactions to these states in about nine thousand experiments on a large number of subjects, the evidence is overwhelming that all emotions and stimuli, whether pleasant or unpleasant, have a very strong tendency to cause vasoconstriction in the peripheral arteries. As the work done by Shepard is absolutely trustworthy, and as he performed one hundred and eighty experiments on two trephined subjects with reliable apparatus, I think his conclusions that all emotions and sensations cause vasodilatation in the brain during the waking state, may be accepted until we have reliable additional evidence to support the opposite view.

Only three experimenters have compared the bodily reactions during a state of tension with those during a state of relaxation. These all found that tension caused vasoconstriction in the peripheral arteries, whereas relaxation caused vasodilatation.

Only two experimenters have studied the vasomotor changes during physical work. Anderson found vasomotor dilatation in the arm in 75 per cent of the reactions from subjects in good physical condition and in but 30 per cent of the reactions from subjects in poor physical condition. Weber found vasodilatation in both the arm and the brain and vasoconstriction in the intestines, when the subject was not tired, and the reverse in the arm and the intestines when the subject was fatigued. There are not sufficient data upon which to base any conclusions as to cerebral and intestinal vasomotor changes during physical work.

The results concerning changes in peripheral volume during mental work are rather contradictory. Most experimenters made no exhaustive study of this problem but requested a few subjects to solve a few

TABLE 1

EXPERIMENTER	DATE	NUMBER OF SUBJECTS	NUMBER OF EXPERIMENTS	FRIGHT	PLEASANT	UNPLEASANT	STIMULI	TENSION	RELAXATION	MENTAL WORK	PHYSICAL WORK
Arm volume											
Anderson	1915–7	30	7000		No difference					+ (−)+ } 75% +25% −75% +50% −50%	+75%
Angell and McLennan	1896				+ (−)	−90%					
Angell and Thompson	1899	2	"Complete"	−	−	−	−				
Berger	1904–7							−		−	
Binet and Courtier	1896			−	−	−	−			≠	
Binet and Henri	1895				−	−	−			−	
Bonser	1903	12			− (+)−	− (+)−				+−	
Citron										−	
Drozynski	1910				+ (−)	−					
Fere	1887				+	−					
Frankfurther and Hir	1909						−			−	
Gent	1903	3	Few		(+) −	−	−	−	+	−	
Gley	1903									−	
Hallion and Comte	1894						−				
Kuppers	1913			− (+)	− (0)	−	− (+)			−	
Lehmann	1892–9	12	100	(+)−	(−)+	−	(+)−(+)	−	+	(+)− −	

Mosso	1879			−	−	−	−			−	
McDougall	1896		1100				−			+−	
Shepard	1905–6	8	289	{ − { (+)−	−	− (+)	−	−	−+	(+)−	
Shields	1895				−	−	−			−	
Sorbonne					−	−					
Stevens	1905		116				{ −80% { + 6%			−57% +29%	
Weber	1910		Few	−	+	−		−		−	+
Weld	1912						−90%				
Wundt								−			
Brain volume											
Berger	1904–7	Few	Few	+−	−	±	+	0		+	
Mosso	1879	3		+	+	+	+				
Shepard	1905–6	2	180	(+)(±)+	+	+	+		+−	+	
Weber	1910	Few	Few			−				+	+
Intestinal volume											
Weber	1910	Few	Few	+	−	+				+	−

+ Increase.
− Decrease.
+− First an increase, then a decrease.
(+)− A temporary increase followed by a decided decrease.
± Now an increase, now a decrease.
$\left\{\begin{matrix}(+)\\ -\end{matrix}\right.$ In some cases an increase, usually a decrease.
0 No change.

arithmetical problems in connection with other experiments. Fourteen experimenters reported vasoconstriction preceded, in a few cases, by a brief vasodilatation. Five, all careful experimenters, found sometimes vasodilatation, sometimes vasoconstriction. Anderson was the only experimenter who found more vasodilatations than vasoconstrictions. His results seem the most trustworthy, however, because he used periods of work long enough to secure true reactions; he performed the same experiment upon thirty subjects and could thereby eliminate individual differences; he experimented upon more kinds of mental work than all the other experimenters combined and he performed several times as many experiments as all the other experimenters combined. We may conclude, therefore, that mental work sometimes causes vasoconstriction in the peripheral arteries, but more often vasodilatation. Weber found that mental work occasioned vasodilatation in the intestinal arteries, and three experimenters found that it caused vasodilatation in the cerebral vessels, as might be expected, since blood always flows to an organ which is being actively used.

Angell and Thompson (3) found that a steady strain of attention caused little if any vasomotor change, and that every break or shift of attention caused vasoconstriction, the amount of constriction being roughly proportional to the intensity of the emotion or stimulus that broke in on the attention, and the amount of surprise involved. My results confirmed theirs, as do the results I have seen everywhere in the literature.

Summary of results obtained by other experimenters. The results of the many experimenters reported in table 1 show that those sensory stimuli (whether agreeable or disagreeable), especially shock, which cause a break or shift of attention, occasion vasoconstriction in the periphery and vasodilatation in the brain, the vasomotor shift being the most noticeable after intense, unexpected stimuli, and the least marked after weak stimuli.

Mental and physical work, which call forth a steady strain of attention, often cause a temporary vasoconstriction in the peripheral arteries due to the shift of attention to the work; then, if the work continues, it brings about a very gradual vasomotor shift, much less noticeable than that occasioned by sensory stimuli, more often vasodilatation than vasoconstriction in the peripheral arteries, and always vasodilatation in the cerebral vessels.

Summary of results obtained by John F. Shepard. In *Organic Changes and Feeling* (29), Shepard reported the results of his experiments on the peripheral volume changes of six normal subjects. Three forms of plethysmograph were employed at one time or another, Zimmerman's modification of Lehmann's plethysmograph, the Hallion-Compte air plethysmograph and the finger plethysmograph described by Lombard and Pillsbury were employed whenever it was possible, and a piston recorder was used with each plethysmograph. The thoracic breathing was registered by means of a Sumner pneumograph connected with a Marey tambour. Among the stimuli used were agreeable and disagreeable smells and tastes, colored lights, deep and shrill whistles, chords and discords on tuning forks, music on the violin and zither, noises, attention to counting marks or a minimal sound, to a touch or to a multiplication, recalling of emotional experiences, listening to amusing reading, etc. The subject's arm rested in the swing which held the plethysmograph. A normal record was run for some time before any stimulus was given and time was allowed for recovery after each stimulus. In some of the experiments the subject and experimenter were in different rooms.

Shepard found that the reaction was almost invariably vasoconstriction, preceded in a very few cases by a temporary vasodilatation. Out of 200 reactions, 187 gave a fall, 7 gave a rise which was probably due to changes in breathing and not directly to the stimulus, and 6 gave a rise followed by a fall, in 3 of which the fall was much more marked than the rise. Twenty-two cases of strain gave a fall in volume; relaxation gave a rise. Agreeable and disagreeable stimuli gave the same reactions.

About 150 clear records were obtained simultaneously of the changes in volume of both the brain and the periphery of a laborer of fully average intelligence who had met with an accident which necessitated the removal of a piece of the skull bone, roughly 8 by 6 cm. in area, on the right side of the head near the Rolandic region. A brain plethysmograph was made by attaching a piece of cork, cut to fit the dip over the trephine, to the rubber membrane of the tambour; this was held on the head by means of two bandages. The same stimuli named above were employed and it was found, in general, that all agreeable or disagreeable stimuli, all sensory attention or attention to arithmetical problems, all agreeably exciting light or music, all depression, and the strain of expectation when a neutrally toned stimulus was announced before it was given, gave a fall of volume of the hand with smaller pulse and more rounded dicrotic, and rise of volume of the brain with larger pulse, often with dicrotic raised in position and made sharper.

With relaxation, there was at first quite often a fall of the hand volume and rise of that of the brain, then a gradual increase of the hand and a decrease of the brain to normal. With strong stimuli, the reaction often had a double character; the volume of the hand increased first with smaller pulse and then fell quickly to a much lower level with rounded pulse while that of the brain increased at first with large pulse, then decreased nearly or quite to normal and sometimes showed almost an anacrotic pulse; then finally rose markedly with high pulse and gradually returned to normal. Similar results were obtained in similar experiments both from this subject and another trephined subject, and were reported in *The Circulation and Sleep* (30).

Experimental literature on stammering. I am acquainted with but one plethysmographic study of stammering. That is an article by Fletcher (49). He had as subjects nine stammerers between 14 and 24 years of age, the average age being 17; there were five boys, three young men and one young lady. No attempt was made to make a careful diagnosis of each case, hence the reader does not know whether these cases were primarily mental or physical stammering. The apparatus consisted of a kymograph, a Lehmann arm plethysmograph connected with a tambour recorder, two Sumner pneumographs for the registration of both chest and abdominal breathing, connected to tambour recorders, a Jacquet chronometer, a Rousselot microphone, a Deprey d'Arsonval galvanometer, an adjustable reading rack and cards on which were printed various selections of prose and poetry. No attempt to minimize arm movements by a swing or otherwise was mentioned, yet stammerers constantly move their arms while stuttering.

The breathing results are trustworthy and were as follows: The characteristic normal rest-breathing curve showed inspirations and expirations of approximately the same length, and the thoracic and the abdominal curves were approximately synchronous in phase. In general, the stutterers presented no permanent peculiarities of breathing unrelated to the function of speech. There were apparently as many varieties of breathing peculiarities among stutterers as there were varieties of stuttering. Breathing abnormalities tended to become stereotyped in certain forms for certain individual stutterers; they appeared often as temporarily adopted expedients to help out in the beginning of speech. The thoracic curve seemed to be more sensitive to mental disturbances than the abdominal curve. In many cases the thoracic and abdominal curves tended to approach each other, often to the point of touching; this sometimes continued throughout the speaking interval. In many cases breathing disturbances appeared before the speaking interval and continued for as long as eight seconds after it. The ratio of the time of inspiration to that of expiration was found to be 0.217 second during normal speech and 0.535 second during stuttering. The expiration interval averaged 24.6 seconds during stuttering, of which the vocalization interval occupied but 9 seconds; the record of normal speech showed that of an average expiration interval of 26.8 seconds, 25.6 seconds were utilized in vocalization. There appeared to be an efficiency of 90 per cent with normal speech as compared with an efficiency of 36.5 per cent with stuttering.

Shepard's plethysmographic results were as follows: There was a marked attention drop (constriction) in 73 per cent of the cases where the stutterer was told to speak or read, this drop being greater in the latter case. Immediately after the attention drop there began, in 90 per cent of the records, a rise (vasodilatation) which usually lasted until the subject ceased stuttering; this amounted to a rise of 5 cm. in 31 seconds in one extreme case. In 62 per cent of the records, this rise was interrupted by irregularities in addition to those due to breathing and movement. When subjects were asked to imagine themselves in situations in which they would be likely to stutter, the curve showed slight vasomotor constrictions in certain cases, never vasodilatation. Both the amount of the general rise and the distortions of the plethysmograph are correlated with the degree of severity of the stuttering.

The speech of the stutterers was attended by an abnormal acceleration of the pulse rate. At a point in the period just prior to the speaking interval, the pulse rate averaged 90.2, ranging from 72 to 120; at a point at the beginning of the speaking interval, the pulse rate averaged 99.8, ranging from 78 to 126; and at a point at the close of the speaking interval, the pulse rate averaged 98.6, ranging from 72 to 129. It will be remembered that the normal pulse rate in adults ranges from 70 to 75.

Apparatus. The apparatus used in these experiments was practically the same as that used by Dr. John E. Anderson, and consisted of a Zimmerman kymograph, a Verdin pneumograph, a Sumner pneumograph, a Marey tambour, a Lehmann plethysmograph, a finger plethysmograph, a piston recorder and two small electromagnets.

The kymograph was the regular Zimmerman model with an extension which permitted the use of smoked paper 2.5 m. long by 16 cm. wide. This kymograph was practically noiseless at the low speed at which it was run. As few of my records required smoked paper over

8 cm. wide, I usually went twice around each record; this enabled me to perform four consecutive experiments of approximately an hour each without changing records.

Zimmerman's modification of Lehmann's arm plethysmograph was used in the earlier experiments. A long-sleeved rubber glove was substituted for the blind sleeve, and a movable but tightly fitting round block of wood was placed vertically within the plethysmograph near the end furthest away from the part where the rubber glove was attached. The plethysmograph was attached firmly to a swing suspended from the ceiling. An elbow-rest, which could be moved along a groove in the swing and clamped at any point, kept the subject from withdrawing his arm; a leather strap, passed over the subject's forearm near the elbow and beneath the swing, kept the subject from raising his arm out of the elbow rest and thus withdrawing it slightly from the plethysmograph; and the vertical stick within the plethysmograph, when lightly clasped, kept the subject from thrusting his arm further into the plethysmograph. Thus the only motion which could seriously affect the record was a motion of the arm within the plethysmograph, a movement which could usually be detected on the record because of its characteristic abrupt rise or fall. The curve often returned quickly to normal after such movements and was then little affected; if it did not return to the normal level, that part of the record was always discarded. The subject was instructed always to clasp the stick lightly, because it was found that there was a tendency to relax the grip now and then when the stick was clasped tightly, in which case the recorder seldom returned to the normal level. He was told, also, to relax his muscles and to keep his hand in as comfortable a position as possible.

After the subject put on the rubber glove of the plethysmograph but before he thrust his entire forearm into the plethysmograph, the operator filled the plethysmograph with water heated a few degrees above bodily temperature and screwed on the glass tube to the top of the plethysmograph. This glass tube was 24 cm. long and, when nearly filled with water, served to press the water tightly against the subject's hand at every point. The subject then thrust his arm into the chamber until he clasped the stick lightly, and the operator adjusted the elbow-rest and strap and added or took water from the glass tube until it was filled to within 2 cm. of the top. The top of this glass tube was connected by thick-walled rubber tubing, through a syringe and outlet to the air, with the piston recorder, as described on page 296 in connection with the finger plethysmograph. The piston of the recorder used with the arm plethysmograph was cut with shears from a thin sheet of brass and was therefore not perfectly round. A strip of bond paper with a fine wire inserted at the outer end was used as a recorder. The lever of the recorder was the same as that to be described later, but it worked up and down between the two springs made of 36 gauge wire (0.019 cm. in diameter) used by Doctor Anderson, touching the springs about 2 cm. from their axes. The lever worked at every point against the delicate springs so that when the pressure against the piston was relaxed, the piston automatically returned to its normal level, thus counteracting the effects of inertia and momentum. By turning the strip of brass to which the springs were attached about the axis, the springs could be set at any distance from the axis of the recording lever; hence the size of the recorded pulse could be regulated for differences in each subject. As this arm plethysmograph could not be used with stammerers, since they moved their arms continuously while stammering, the finger plethysmograph was substituted in my later experiments, in order that I might compare more readily the vasomotor changes in stammerers with those in normal speakers.

The finger air plethysmograph was made in the shop at the laboratory and was a modification of the one used by Lombard and Pillsbury and described in volume III, pages 191–193, of this Journal. The air chamber consisted of a brass pipe 10 cm. long and 2.5 cm. in diameter with a metal disk, pierced by a pipe with a bore 4 mm. in diameter soldered to one end, and a rubber diaphragm 0.026 inch thick, with a small hole in the center, fastened to the other end. This air chamber was placed inside of, and projected 1 cm. in front of, a second watertight pipe, 12.5 cm. long and 4.5 cm. in outside diameter, with two outlets through which water of constant temperature might pass and keep the air in the air chamber at constant temperature. As there was no running water in the room, the water chamber was not used. This plethysmograph was firmly attached to a swing suspended from a rod 43 cm. above it. When the subject thrust the middle finger of his right hand through the hole in the rubber diaphragm until it could go no farther, the operator adjusted the same elbow rest and strap used with the arm plethysmograph. Then no ordinary movements could affect the record other than movements of the finger within the plethysmograph; these could usually be readily detected because of their characteristic abruptness. As the recorder usually returned to its normal level after such movements, they seldom affected the record;

the few parts of records in which the lever did not return to its normal level were discarded. A thick-walled rubber tube connected the finger plethysmograph with a metal T-tube. One end of the T-tube led to a glass syringe 10 cm. long with a piston 13 mm. in diameter; the other end of the T-tube led through a thick-walled rubber tube of the same diameter to a second similar T-tube. One end of this second T-tube led to the open air and was always open when no experiment was in progress, but always closed by a metal clamp when the experiment was in progress; and the other end led to the piston recorder. As the syringe was airtight, it was possible, by moving the plunger, to alter the amount of air in the system of tubes connecting the finger chamber with the recorder and thus place the lever in a horizontal position when it was working too high or too low because of changes in temperature or arm movements.

Of the many forms of apparatus which have been devised for recording minute vasomotor changes, a piston recorder, properly made, is the most delicate. As the piston recorder which I used with the arm plethysmograph made no record when attached to the finger plethysmograph, it was necessary to modify this recorder.

I selected a glass T-tube having a bore 5.2 mm. in diameter which was almost round. One of the opposite ends was sealed; the other was ground or lapped out so as to be perfectly round its full length for the piston to work in. A perfectly round piston, which fitted the piston barrel closely and yet worked up and down and turned almost over in the tube without binding, was cut from a strip of celluloid 0.026 inch thick. The celluloid piston was practically as stiff as one made of sheet brass, yet soft enough to be cut by a hollow round steel punch. This was a decided advantage because brass has to be cut with shears and will necessarily have little corners which will permit air to escape and injure the record. The point of a stiff steel pin, 0.028 inch in diameter, was passed through the center of this piston and slightly riveted so that it could not pull off; the head was cut off and a hook was made of this end. When this hook was passed through a hole which was drilled in the brass lever and which just fitted it, this pin made a very satisfactory piston rod 4.5 cm. in length.

The lever of the piston recorder was the one used by Doctor Anderson, excepting that his writing lever made of bond paper tipped with a fine hair was replaced by a very thin strip of celluloid, 0.01 inch in thickness, which was shaped at one end to form a sharp writing point and was attached by varnish to the sheet brass part of the lever. The celluloid lever was almost as flexible horizontally as the bond paper but it had greater vertical rigidity, could not be bent by accident as could the paper and was not affected by air currents as was the paper. All of the lever but the celluloid tip, which was 54 mm. long and 4 mm. wide, was cut from a piece of sheet brass 0.018 inch thick. It was made from a strip 13 cm. long and 4 mm. wide, and one end was turned twice at right angles and fastened to its axis by the pin *MN* as shown in figure 1. A small piece of lead, *L*, was placed on the arm of the lever on the opposite side of the axis from the writing point; thin shavings were cut from this lead until the piston would stay at any point of the barrel where it was placed, before oil was dropped into the barrel or the writing tip touched the drum of the kymograph. Thus the pressure on the lower part of the piston had to work only against the weight of the drop of heavy machine oil on the top of the piston and the friction of the joints and of the writing tip of the lever on the smoked paper; this proved just sufficient in an upward thrust to diminish the extra upward movement due to momentum from the strong upward thrust at the beginning of each pulse beat. As the weight of the drop of oil and the friction on the smoked paper act in opposite directions when the piston is falling, the lever quickly returned to normal after each pulse beat. I watched the piston closely during all experiments and seldom saw a bubble of air escape unless a little oil hardened on the sides of the piston-barrel and did not permit the piston to move freely. I avoided this by frequently removing with alcohol the oil which is bound to adhere to the sides of the barrel as the piston works up and down. What little oil ran down the sides of the tube collected at the sealed end directly below the piston-barrel and did not interfere with the pulse waves which came in through the third horizontal arm of the T-tube from which the recorder was made. The piston could move an inch above or below the position it assumed when the lever was horizontal, without catching or binding.

The springs used with the arm plethysmograph were discarded as the changes in pressure due to the minute vasomotor changes in a single finger were not sufficient to move them. The writing point was 12.5 cm. from the axis of the lever and the piston rod was attached to the sheet brass part of the lever 3 cm. from the axis; hence the motion of the piston was magnified a little more than four times on the record. When the piston rod was attached nearer to the axis of the lever, the piston was apt to bind in its barrel, and the piston rod often touched the sides of the barrel and bound when the lever moved much above or below the horizontal position. If the lever were much longer, the friction between the writing tip and the drum would increase and interfere with the free working of the piston within its barrel.

The piston recorder was fastened securely to the end of the round horizontal rod *AB* by the clamp *A*. This horizontal rod was fastened by a clamp (not shown in the figure) to a vertical rod which was firmly attached to the horizontal revolving arm of a stand which could be raised or lowered by turning a screw, and placed nearer or farther away from the drum of the kymograph by moving it gently with the hand. At a convenient distance from the end of the horizontal rod *AB*, a vertical hole, *B*, was drilled to fit the smaller round rod *BQC* which was turned at right angles at *Q*. Two horizontal holes, *C* and *D*, were drilled at right angles to each other in the short square rod *CD* to fit the round rods *BQC* and *DEFG*. Rod *DEFG* was turned horizontally at right angles twice at *E* and vertically at right angles at *F* and was split down the middle and bent at *G* to hold the axis pin *MN*. Screws clamped the rods firmly together at *B*, *C* and *D*. The piston could be raised or lowered to any point of its barrel by loosening the screw *B* and raising or lowering the vertical rod *BC*. The piston rod could be brought over the center of the piston barrel and made parallel to this by loosening screws *C* and *D*, moving the rod *DEFG* and revolving it about the axes *QC* and *DE*. When the lever is horizontal, the apparatus should be so adjusted that the piston-rod will lean

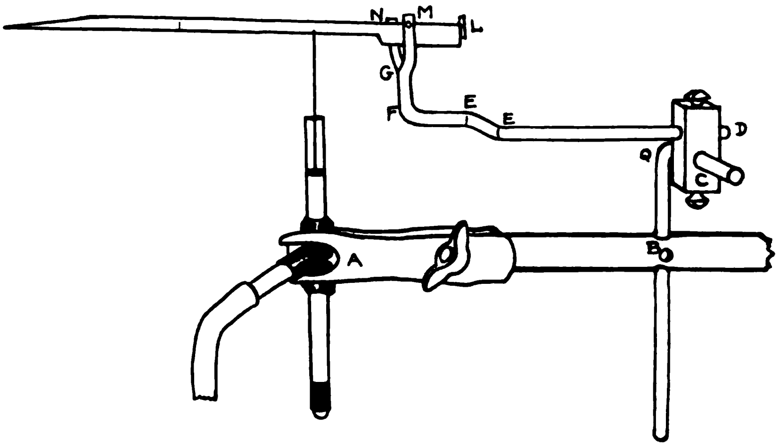

Fig. 1

slightly toward the writing point because it swings away from the writing point with either an upward or downward movement, and such an inclined position keeps the average position of the piston-rod more nearly vertical and prevents binding.

This piston recorder satisfied the requirements outlined by Lombard and Pillsbury on page 187 of volume III of this Journal. The piston was thin so as to minimize the friction in the piston barrel. It was of small diameter and so connected to the piston rod and the lever that it could not bind and could tilt but little. The movable parts were as light as possible in order to avoid the effects of inertia and momentum. The piston moved freely in the tube and yet followed readily the movement of the lever, which not only described an arc as it moved up and down, but moved a little to one side or the other when the writing point passed over an uneven place on the smoked paper, so as not to keep exactly over the center of the piston. As the compressibility of the air below the piston requires that the lever should write on the smoked surface of the kymograph with the least possible friction, the lever was made flexible so as to yield to the inequalities in the recording surface and at the same time be sufficiently springy to maintain a continuous, gentle pressure on the drum of the kymograph.

The lever of the piston recorder worked up and down in a vertical plane parallel to the drum of the kymograph. The writing point should be tangent to the drum when the piston is about 15 mm. above or below the position it assumes when the lever is horizontal. If the writing point is tangent when the lever is horizontal, it will not, when it rises or falls, touch the drum more than a few millimeters, unless, when horizontal, it presses too tightly against the drum to make an accurate record.

To test the effect of this change in pressure on the drum as the writing lever approaches and recedes from the drum, and to see what change in volume of the finger corresponds with the rise or fall of 1 mm. on the record, I constructed the following piece of apparatus. With thick-walled rubber tubing I connected one end of a glass capillary tube, 10 cm. long, with a bore 2.25 sq. mm. in cross section, with the part of the T-tube which led to the air in my former experiments. I attached about 30 cm. of the same rubber tubing to the other end of the capillary tube and passed it between a board and the table. I clamped the capillary tube in a vertical position close to a scale and filled it with mercury. By tightening or loosening the clamp which pressed the board against the table, I could raise or lower the height of the mercury in the capillary tube. As I made the volume of air from the mercury to the T-tube the same as that in the tube which formerly led to the air, and placed in the plethysmograph a wooden finger of the same volume as my own finger, the volume of air in the plethysmograph and connecting tubes was the same as it was in my former experiments; the wooden finger, of course, could not move. When this apparatus was connected, I lowered the mercury by eight equal amounts and then raised it by the same number of equal amounts, running the kymograph for an instant between each rise or fall. I did this several times. Then I measured the height of each little rise or fall on the smoked paper and found that these all averaged the same length for a given position of the lever, and that the position of the writing lever therefore made no difference in accurately recording the vasomotor waves when it just touched the drum when the piston was raised just a little more than 15 mm. above the horizontal position of the lever. When the writing point just touched the drum when the piston was raised considerably more than 15 mm. above the horizontal position of the lever, the pressure of the writing point on the drum became so great, when the lever was horizontal, that the mark then traced was much shorter than that traced when the writing point just touched, although both represented the same change in volume. It is necessary, therefore, to move the writing point nearer the drum if the writing point rises or falls much more than 15 mm. from the horizontal position, and to move it away the same amount when it returns.

I repeated this procedure when the plunger of the syringe was at one end of the syringe and again when it was at the other end, and found that no difference in amplitude could be detected in the record for like changes in the column of mercury at these two positions; hence the differences in the position of the plunger of the syringe in different experiments, and differences in the sizes of the fingers of different subjects which were in all cases less than the volume of the syringe, do not affect a trustworthy record of the vasomotor changes.

I found that a rise of 1 mm. on the records always denoted an increase in volume of the finger of 3 cu. mm., and that a fall of 1 mm. on the record always registered a decrease in finger volume of 3 cu. mm., for changes not exceeding 15 mm. above or below the horizontal position of the lever, and for all changes if the lever is moved a little toward the drum when the rise or fall exceeds 15 mm. and is withdrawn the same amount when it returns to that point.

As my piston recorder appeared to move instantly when I moved the plunger of the syringe, and as the latent period of a similar piston recorder tested by Lombard and Pillsbury was found to be but one one-hundredth of a second, I did not repeat their test. A latent period of less than a second would not affect my results.

As Lombard and Pillsbury found by careful experiment that the curve traced on the record by a piston recorder similar to mine was a true record of the normal pulse curves from the finger, and does not originate in the recording mechanism, I shall assume the same to hold true with my recorder without repeating their elaborate test experiment.

The thoracic breathing was obtained either by a Sumner pneumograph or by a Verdin pneumograph. The connecting chain was passed just below the subject's armpits. As the Verdin pneumograph frequently turned over during the stammerers' contortions and gave a less magnified record than the Sumner pneumograph, the Sumner pneumograph was used in practically all of the records so that the records of normal speakers and stammerers might be more easily compared.

The breathing record was obtained by a writing lever attached to a Marey tambour 2.5 cm. in diameter. The lever was well balanced and consisted of a bond paper writing arm with a very fine wire at its outer end. As the tip of the writing wire was 14.3 cm. from the axis of the lever, and the lever was attached to the rubber diaphragm of the tambour 1.2 cm. from the axis of the lever, the movements of the tambour were magnified twelve times. As the rubber diaphragm of the tambour was uppermost in my earlier experiments, the top of the curve in these records represents empty lungs. As the rubber diaphragm was inverted in my later experiments, including most of the work with the finger plethysmograph, the top of the curve represents full lungs in these records; this seemed more logical and made the records of the stammerers easier to read. Unless specifically mentioned to the contrary, the top of the records here reproduced will represent full lungs and the bottom of the records empty lungs.

The breathing record is a valuable check on the plethysmographic curve as it shows what sudden changes in the plethysmographic record are caused by deep breathing, yawning, laughter or sneezing. The breathing record was especially useful in comparing the breathing of stammerers and normal speakers while reading the same passage. I used the pneumograph only for this purpose.

A thin celluloid writing lever, pointed at the outer end, was attached to each of the electromagnets. The upper magnet was connected with a two-way switch. One side of this switch was connected with a time key which could be pressed by the operator and with another key which could be pressed by the subject. The other side of the switch was connected with one side only of a large electric pendulum making one stroke a second; this caused the magnet to click and mark the record every two seconds. When the clock happened to be out of order, I used a stop watch and made marks on the record at known intervals by pressing the operator's time key; as the kymograph rotated at practically a uniform rate, the time could be interpolated pretty accurately between any two points on the record. The lower electromagnet was connected *a*, with the operator's signal key; *b*, to a key which could be pressed by the subject; *c*, in series with a signal light in front of the subject through a switch near the operator and *d*, in parallel with a faint signal buzzer near the subject through another switch near the operator; thus signals were recorded on the record the instant they were given, unless the operator had to use one hand to make the stimulus, as in blowing a whistle, and press his signal key with the other hand, when there was apt to be an error of a fraction of a second. Such an error, however, was too small to influence the results, as I did not try to make any measurements in fractions of a second, whole seconds being accurate enough for the purposes of these experiments.

Procedure. The arrangement of the apparatus used is shown in the accompanying photograph. The writing points of the four recorders were adjusted to write in the same vertical line when the recording levers were horizontal. The time recorder was at the top, the operator's signal recorder next below, the tambour recorder which traced the breathing curve below that and the piston recorder, which traced the vasomotor changes, was at the bottom. I adjusted the swing at a comfortable height for the subject's arm and then adjusted the elbow-rest. Next, when the subject's lungs were half full of air, I put on the pneumograph so that its writing lever would be above the horizontal position as much of the time as it was below it, and thus work more nearly straight up and down. After requesting the subject to remain quiet until the experiment was over, I then closed the outlet from the tube which connected the finger plethysmograph with the air. When the writing point of the piston recorder remained still for a few seconds, showing that the temperature within the tubing had ceased to rise noticeably, I started the kymograph. As I was interested in the volume changes rather than in the shape of the pulse, I ran the kymograph at low speed; hence little, if any, pulse waves are noticed in these records. Very distinct pulse waves appeared in some subjects, however, whenever the kymograph revolved at high speed. I ran the kymograph for about a minute before any stimulus was given, in order to obtain a normal record from which to measure the reactions to the stimuli that followed; during this normal period I asked the subject to remain as calm and as quiet as possible so that no emotions or movements would affect this normal reference line. After the stimulus was given, at least a minute was allowed for the piston recorder to return to its normal level, unless it returned much sooner. Sometimes several stimuli were given in quick succession; sometimes stimuli were given at intervals of a few minutes; sometimes but one or two stimuli were given in an entire period of an hour. The kymograph was stopped at intervals to secure reference lines from the four recorders, to make sure they were keeping in a vertical line. The subjects were allowed to rest and to move whenever they became tired

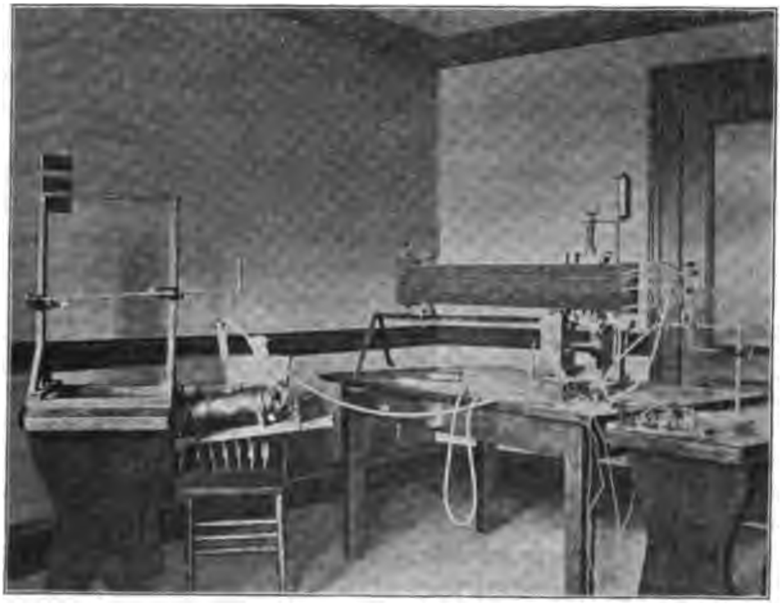

Fig. 2

or uncomfortable or whenever their records showed frequent arm movements; but first they always asked the experimenter's permission to move. A screen was always placed between the experimenter and the subject so that the subject could not see what stimulus was next to be given and the operator was careful not to make a noise when handling stimuli.

I had, as subjects, thirteen graduate students or college seniors and ten stammerers. The normal speakers were experimented upon once each week at the same hour, between 9 a.m. and 1 p.m.; three came for two months, six came for four months and four came for eight months. Nine were men, four were women. The stammerers, all young men with the exception of one boy, came at irregular intervals, frequently in the evening after a hard day's work; some came but once or twice. I selected from about one hundred students at the Boston Stammerers' Institute those who could be depended upon to stammer whenever they were called upon to read and those who did not move their arms violently while stammering; a large majority of the pupils could not be experimented upon.. As most of the pupils experimented upon learned to read without hesitancy within two weeks from the time they entered the Institute, they could not long be experimented upon as stammerers. Some came to the laboratory at various intervals during their cure, so that I could study the vasomotor changes at different parts of their cure and compare them.

I gave, in all, about 1000 stimuli. A large majority of the records had to be discarded because they were obscured by arm movements. Less than 1 per cent of the reactions to stimuli with the Lehmann plethysmograph were comparatively free from arm movements. About 150 reactions to stimuli were retained and measured.

I employed the following method to obtain the fall or rise in millimeters of the recording needle during an emotion, the time in seconds from the stimulus to the lowest or highest point of the curve traced immediately after the stimulus was given and the time in seconds from this lowest point to the return of the needle to normal. I placed a transparent celluloid triangle over the record and moved it around until I got one edge over what seemed to be the route the needle would have taken if the stimulus had not been given, allowing for all arm movements as far as possible. If I did not wish this part of the record for reproduction, I then drew a line with a fine needle along this path which the recording needle would have traced if no stimulus had been presented; if I wished to preserve the record for publication, I left the triangle lying there. The line traced by the needle was never parallel to the time line, for the tube was usually either warming up a little or cooling down a little even when I tried to keep the room temperature constant. The lowest or highest point of the curve was obtained by placing a second triangle touching the first before it was moved from this position, and sliding the first triangle along the second until it became tangent to the lowest or highest point of the curve. This position of tangency was marked on the record with a sharp needle. I next placed a triangle along reference lines taken at intervals on records to ascertain whether the ends of the piston recorder, tambour recorder and two electromagnet recorders were keeping in a straight vertical line, so as to be tangent to the reference marks traced by the operator's key and the piston recorder. I placed a second triangle touching the first and slid the first along the second, marking on the time line the point corresponding with the instant the stimulus was given, the point corresponding to the moment when the fall of the needle was at its maximum and the point corresponding with the instant when the needle returned to normal. By counting the time notches between these marks, I obtained the two times referred to above. By placing one leg of a right triangle on the time line with the other leg passing through the lowest point of the plethysmogram, I was able to measure along the latter leg, with a millimeter scale, the drop to the lowest point of the curve from the standard line the needle would have traced if no stimulus had been given. If an arm movement was noticed and the needle did not return to this standard line within a minute from the time the last stimulus was given, that part of the record was discarded. Arm movements could usually be readily distinguished because of their abruptness. If a record contained arm movements at intervals of an inch or two, I discarded that part of the record, because these movements showed that the subject was then too nervous to keep still, so that it was impossible to get an accurate reference line.

Among the stimuli employed at one time or another to arouse emotions in both normal speakers and stammerers were the following:

1. A shrill whistle.
2. A loud crash, sometimes in front of the subject, sometimes behind him.
3. A sudden yell from the operator.
4. The report from a cap, sometimes placed in the book the subject was asked to read so that it would go off when he opened the book.

5. The sudden entrance of a third person into the room.

6. A loud sneeze by the operator.

7. The subject read silently an exciting story or gruesome description and pressed a key whenever he experienced an emotion.

8. The operator let down an artificial spider on a string close to the subject's head.

9. The subject was directed to take an unpleasant unknown object, such as a rubber snake, out of a cloth bag.

10. The operator threw a light, artificial rubber snake at the subject.

11. The operator aimed at the subject a noisy, self-propelled red balloon.

12. The operator placed close to the subject material for a bonfire, oiled it in his presence and told him to open the match box given him at a signal and light the fire. (When the subject pressed the catch to open the match box, a spring made it fall apart.)

13. A large mechanical bug crawled toward the subject.

14. The operator set near the subject a mechanical frog adjusted to jump six feet into the air about half a minute after being placed.

RESULTS

As arm movements affected most of the curves traced by the piston recorder when it was attached to the arm plethysmograph, no records made with the arm plethysmograph are included in the following tables. Although these curves could not be accurately measured and compared, they confirmed the results tabulated below.

All of the curves showed vasoconstriction in the finger both during stammering and during the emotional disturbance induced by a loud, sudden stimulus such as a shrill whistle, which will hereafter be called *shock*. A small proportion of the curves showed a slight rise for three or four seconds, followed by a decided fall of considerable duration.

In all of the tables, "fall in millimeters" was measured from the path the writing needle of the piston recorder would have taken had no stimulus been given, to the lowest point of the curve immediately following the stimulus. If, during a long period of stammering, there was a temporary rise followed by a greater fall than the first one, the greater drop was the one measured. Figures 4 and 6 show the method of measuring these falls. "t" was measured in seconds from the instant the stimulus was given to the time when the recording needle first attained the maximum drop. "T" was measured in seconds from the end of "t" to the time when the recording needle first returned to the path it would have taken had no stimulus been given, unless the time of maximum drop occurred during a long period of stammering, when "T" was measured from the end of the stammering period instead of from the end of "t." Under each of the headings "fall," "t" and "T," the maximum, the average, and the minimum are given, so that individual differences may be studied.

Table 2 shows the vasomotor changes in seven normal speakers during shock induced by the six kinds of stimuli named in table 3. These subjects are listed in the order of decreasing magnitude of fall. This table shows that these subjects are also arranged approximately in the order of increasing length of "t," but in the order of decreasing length of "T" the correlation between the average "Falls" and "t's" being −0.775, and the correlation between the average "Falls" and "T's" being +0.908. The order is the same, in practically every case, both for the maximum and for the average. This table shows, therefore, that for the subject who has a relatively large fall, the time of fall is relatively short; for example, subject E's average fall of 21 mm. occurred in an average of 10 seconds, whereas subject D's average fall of 4 mm. occurred in an average of 32 seconds. This table shows, furthermore, that those subjects who experience the largest falls also have the slowest recovery and the individual cases show that the greater the fall,

TABLE 2

Vasomotor changes in individual normal speakers accompanying shock

SUBJECT	NUMBER OF STIMULI	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
		Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
E	5	26	21	15	16	10	5	100	56	33
G	15	20	13	5	22	11	2	95	35	7
H	12	19	10	5	43	14	3	98	36	13
A	9	13	8	5	44	23	6	75	47	16
J	13	15	8	3	28	14	8	98	39	10
F	9	20	6	2	70	31	7	64	31	11
D	4	9	4	1	51	32	23	64	37	12
Total	67									
Average		17	10	5	39	18	8	85	39	15

the slower is the recovery. A study of the individual cases shows that subjects do not vary any more from day to day than they vary during a single hour. As table 3 indicates, these differences for the same subject are due to the intensity of the stimuli and to the degree of surprise involved. All these tables show decided individual differences.

TABLE 3

Vasomotor changes in normal speakers accompanying shock brought on by stimuli of different intensity

STIMULUS	NUMBER OF STIMULI	NUMBER OF SUBJECTS	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
			Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
Gun	5	4	26	18	7	34	14	2	100	63	10
Whistle	10	5	18	9	5	24	12	6	80	30	15
Threw snake	5	3	15	10	6	38	14	6	60	40	13
Crash	19	7	23	9	1	51	19	2	98	34	11
Yell	3	3	7	6	5	70	31	10	40	29	23
Spider	7	5	11	7	2	23	15	6	72	31	12
Total	49	7									
Average			17	10	4	40	17	5	75	36	14

TABLE 4

Vasomotor changes in stammerers accompanying shock brought on by stimuli of different intensity

STIMULUS	NUMBER OF STIMULI	NUMBER OF SUBJECTS	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
			Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
Gun	7	5	50	25	9	16	10	5	110	80	38
Whistle	11	5	30	19	8	20	12	7	88	44	19
Threw snake	5	5	30	15	0	19	13	9	65	48	37
Crash	6	5	23	14	9	65	21	6	93	50	25
Yell	6	4	25	16	10	19	12	8	58	43	30
Spider	4	4	13	8	3	11	7	4	34	28	21
Total	39	10									
Average			28	17	6	25	13	6	74	50	28

Table 3 shows the reactions of the seven normal speakers listed in table 2 to six stimuli of different intensity. Table 4 shows the reactions of ten stammerers to the same stimuli. These tables show that the more intense the stimulus is, the greater is the vasoconstriction and the slower is the recovery. A close examination of the individual curves showed that the more unexpected the stimulus is, the greater is the vasoconstriction and the slower is the recovery; this is seen when the reaction to the first gun fired is compared with that to later reports of the same gun, or when the whistle is blown several times on the same morning and the successive reactions are compared, or when the introspections of the subjects tell which records to compare. These tables also show that stammerers as a class experience greater and more rapid vasoconstriction and slower recovery in shock than do normal speakers.

TABLE 5

Vasomotor changes in individual stammerers accompanying shock

SUBJECT	NUMBER OF STIMULI	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
		Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
Web	7	50	26	8	11	9	5	100	59	29
Wi	7	30	23	9	12	10	7	110	66	35
Ad	4	23	20	14	16	15	11	93	65	30
De	3	23	14	8	20	19	19	58	41	19
Li	5	18	12	10	9	8	6	65	42	30
An	4	15	12	10	14	10	7	75	38	22
Fa	1	12	12	12	65	65	65	25	25	25
Bo	2	8	6	3	13	12	11	42	34	27
Total	33									
Average		22	18	9	20	13	16	71	52	27

Table 5 shows the reactions of eight stammerers to shock produced by the six stimuli listed in table 4. This table, like table 2, shows that those stammerers who experience a relatively great vasoconstriction also require a relatively long time for recovery, the correlation between the average "Falls" and "T's" being +0.698. It shows, also, that those subjects who experienced relatively great vasoconstriction tend, like normal speakers, to have relatively rapid vasoconstriction, although the correlation between the average "Falls" and "t's" is but −0.039. This is not so striking as for the normal speakers, because fewer stimuli were given and the stammerers had not been trained to keep their arms still, as had the normal speakers; nevertheless this relationship is noticeable in the first four subjects listed. The stammerers experienced 80 per cent greater vasoconstriction than did the normal speakers, and this attained its maximum in two-thirds the time it did in normal speakers. The time of recovery was 23 per cent longer for the stammerers than for the normal speakers. The maximum fall for stammerers was almost double the maximum fall for normal speakers, and the minimum time of recovery was nearly three times as long for the stammerers as it was for the normal speakers. Shock, therefore, is a greater emotional disturbance in the average stammerer than it is in the average normal speaker. An examination of the individual reactions shows clearly that the greater the vasoconstriction is, the slower is the recovery in practically every case. The relation between the magnitude of the fall and the rapidity of the fall is not so definite when we study the individual curves of normal speakers and stammerers. In about half of the subjects, the time of fall is about the same for both large and small falls; in others it is roughly proportional to the fall, in others it is roughly inversely proportional to the fall, and in still others there is no relationship whatsoever.

Table 6 shows the vasomotor changes of the eight stammerers listed in table 5 during stammering. For these eight stammerers as a class, the average drop was 20 per cent greater in shock than in stammering and the average time of recovery was 2 per cent longer than in stammering. For stammerers as a class, therefore, stammering is almost as great an emotional disturbance as shock. If the curves of shock caused by the shots are disregarded from the results it will be seen that stammering produces a greater emotional disturbance than shock, for the average fall is exactly the same and the time of recovery is 16 per cent longer in stammering than in shock. It will be noticed that the subjects are arranged in both table 5 and table 6 in the order of decreasing magnitude of the fall, and that the subjects do not occupy the same places in the two tables. Thus Bo, who was last in table 5, is practically first in table 6. Web, who occupied first place in table 5, has the last place in table 6. With the exception of Fa, who reacted but once to a single stimulus and who can therefore hardly be assigned to a relative postion in table 5, the other subjects occupied about the same places in the two tables. It is clear that the subjects are not in reverse order in the two tables. It simply happened that Web was the slightest stammerer of the eight and was greatly affected by shock, and that Bo, a senior medical student, was one of the severest stammerers of the eight, and was little affected by shock. The correlation between table 5 and table 6 is high, being +0.919 for the "Falls" and +0.897 for the "T's," if Fa is excluded.

A study of the individual records shows that the time of recovery bears no relation to the length of the passage read but is dependent, rather, on how severely the subject stammered while reading the passage, especially at or near the end of the passage. If, in the middle of the passage, the stammerer got a good start, he stammered little and the needle began to rise; if he then met a hard word and either became afraid of it or began to stammer severely, the needle fell; if such a hard word occurred near the end of the passage read, the needle had further to rise, and "T" was lengthened. The time of recovery never occurred during a stammering interval, and it occurred but twice at the end of a stammering interval—once, after a subject who never stammered while reading after his eighth lesson had been reading aloud, and once when the maximum drop occurred more than a minute before the end of a four-minute reading period in a curve where it was difficult to obtain an accurate reference line. In only two other cases did this recovery take place within twenty seconds; twice Web, the slightest stammerer in the group, recovered in 16 seconds. The recovery was accomplished in from 20 to 25 seconds in nine cases, seven of these being the curves of the two slightest stammerers; in from 25 to 30 seconds in six cases; in from 30 to 40 seconds in six cases; in from 40 to 50 seconds in eight cases; in from 50 to 60 seconds in seven cases; in from 60 to 80 seconds in six cases; in from 80 to 100 seconds in six cases; and in more than 100 seconds in four cases. Thus the recovery

TABLE 6

Vasomotor changes accompanying stammering

SUBJECT	NUMBER OF STIMULI	LENGTH OF STAMMERING	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
			Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
Wi	9	60	36	21	12	165	72	24	133	73	42
Bo	7		30	20	11	110	80	20	129	85	40
Fa	6		19	16	14	48	40	25	55	39	20
De	7	121	21	15	9	120	65	40	82	60	34
Li	2		17	12	6	42	37	32	75	48	21
Ad	8	36	17	12	7	54	24	8	80	40	21
An	8	89	18	12	8	94	63	27	48	29	0
Web	6	54	21	11	4	60	25	7	30	23	16
Total	53										
Average		71	22	15	9	87	53	23	79	51	24

occurred within twenty seconds in but 7 per cent of the stammering curves, and then only in the plethysmograms of the two slightest stammerers and in one other which was in doubt. It took place in from 20 to 30 seconds in 27 per cent of the curves, 73 per cent of these curves being those of the three slightest stammerers. It was completed in from 30 seconds to 1 minute in 37 per cent of the cases, and in more than 1 minute in 29 per cent of the cases. Eighty-one per cent of the latter were the curves of subjects Bo, De and Wi, who were among the four severest stammerers. The worse a person stammers, therefore, the slower is the recovery.

Before compiling these tables, I arranged the eight stammerers in the order of decreasing severity of impediment as follows: Fa, Wi and De, Bo and Li (in doubt, as neither had completed his course at the Boston Stammerers' Institute), Ad, An and Web. It will be seen that the order in table 6 is very much like the order in my estimate as to the severity of the several cases, the correlation being +0.79; hence the amount of vasoconstriction and the time of recovery are important factors in determining the severity of an impediment of speech. As some subjects are more sensitive than others, the ratio of time of recovery from stammering to the time of recovery from shock is a more accurate estimate of the severity of a case, a ratio more than 1.35 being a severe case, a ratio of unity being about an average case and a ratio less than 0.65 being a moderate case. According to this ratio of recovery times, the following order of decreasing severity of impediment is obtained, Bo...2.50, Fa...1.56, De...1.46, Li...1.14, Wi... 1.11, Ad...0.62, An...0.61 and Web...0.39, the correlation with my estimated order being +0.76. If another, similar ratio is found, the ratio of the average fall accompanying stammering to that accompanying shock, the following is the order of decreasing severity of impediment: Bo...3.33, Fa...1.33, De...1.07, Li...1.00, An...1.00, Wi... 0.91, Ad...0.60 and Web...0.42, the correlation with my estimated order being +0.61, and the correlation with the time of recovery ratio being 0.90. The order is the same in the time ratio and the fall ratio with the exception of An, who comes two places earlier in the latter than in the former. As the magnitude of the fall is affected by the varying pressure of the writing needle on the drum and by finger movements, the time of recovery ratio is the more accurate of the two.

A study of the individual plethysmograms shows that if a stammerer who has been taught to read without hesitancy attempts to read when the peripheral vasoconstriction from shock is at its maximum, he will stammer severely; if he reads the same passage a minute later after this constriction has ceased, he does not stammer. If a normal speaker is about to speak a word when a shrill whistle is blown, the sound of that word is suddenly lost and he is unable to say it until peripheral vasoconstriction has ceased; that is, he becomes a temporary stammerer. The typical stammering curve remains low throughout the interval of stammering, with occasional temporary increases and decreases in the low volume. When stammerers are partially cured, the amount of peripheral vasoconstriction decreases and the time of recovery decreases. When the stammerer is cured, his record shows no drop unless it has the same brief attention drop that regularly accompanies other shifts of his attention. If stammering begins with an attention drop as normal speech does in some subjects, the stammering curve will remain low throughout the stammering interval while the normal speech curve will begin at once to return to normal and will sometimes rise above normal as shown in figure 9. Subjects who give an attention drop when they commence to read or speak are apt to give a smaller one when they stop reading or speaking. This delays the recovery in both stammering and normal speech; it is more noticeable on the normal speech curve because the curve is already low on the stammering plethysmograms. Less than half of the subjects show a decided attention drop; those who give it for reading also give it for every shift of attention; hence it can readily be distinguished as an attention drop.

Most of the stammerers who served as subjects had no fear of stammering in my presence and hence could not imagine themselves in embarrassing surroundings. Those who were able to imagine themselves in tight places were told that they would be called upon to introduce two persons or to buy a prescribed article at a store in a given number of seconds. During this period of suspense, Web averaged a drop of 11 mm. in an average of 14 seconds and Wi averaged a drop of 19 mm. in an average of 41 seconds. This shows that the fear of stammering causes marked vasoconstriction even when a stammerer is making no effort to speak.

It is useless for a stammerer to attempt to speak without hesitancy until peripheral vasoconstriction has ceased; hence he must wait at least twenty seconds before he commences to speak and should wait nearly a minute if he is a severe stammerer.

It will be seen that my results do not agree with Fletcher's. Fletcher found that in 90 per cent of his records a rise in the plethysmogram began immediately after the initial attention drop and usually lasted until the end of the stammering period; I found that, with very few exceptions, the curve remained low from this attention drop to the end of the stammering period. Fletcher has published only a portion of two curves; the rise is not conclusive in either. In his plate D, the writing point which traced the pneumogram kept interfering with the writing point which traced the plethysmogram throughout the rise, lifting it forcibly in several places. In his plate E, there is no normal reference line to go by; the curve is cut from the middle of a period of stammering, and no normal curve is shown either before or after the subject stammered. The curve looks like some of mine that were traced immediately after the subject placed his finger in the plethysmograph when the air in the tubing was still warming enough to expand and cause the recording needle to rise steadily; I suspect that either the room or the air in Fletcher's connecting tubes was warming, causing the air in the tubes to expand and raise the writing point, and that the same rise or a greater one would have occurred during reverie. I frequently found gradual rises due to slow movements in the finger after attention drops, especially in shock; these gradual movement rises were always much steeper than the general rise going on at the same time, and ended in a gradual drop followed by a gradual return to the normal level in approximately that straight line, which, if produced, would pass through the point where the movement rise first began. If these gradual movement rises, due to involuntary, slow tightening or relaxing of the finger lasted for several seconds, it is reasonable to suppose that they would last longer in the arm and that Fletcher may have mistaken them for vasodilatation when he happened to stop the kymograph before the movement ceased and the curve began to fall. I used the Lehmann and the finger plethysmographs simultaneously on one subject and found that when the finger plethysmograph's recorder showed vasoconstriction, arm movements within the Lehmann plethysmograph often caused its recorder to register vasodilatation; the abrupt nature of these rises showed that they must be due to arm movement. All stammerers continually move their arms while stammering but seldom realize the minor movements. These movements affect the Lehmann plethysmograph much more than they affect the finger plethysmograph; it was for this reason that I discarded the former in favor of the latter. I think Fletcher expected a rise and was therefore prejudiced when tabulating his results. He may have discarded curves in which there was a fall, believing this fall was caused by arm movements. Both Fletcher and I found that the distortions of the plethysmograms are correlated with the severity of the stammering; I found, however, that the amount of the general fall, rather than of the general rise, was correlated with the severity of the stammering.

My results also confirm Fletcher's statement that the curve showed no positive changes, and showed in certain cases a slight decline when the subjects were able to imagine themselves in situations where they would be likely to stammer; my subjects who not only imagined themselves in such situations, but actually lived over again such occasions, gave a marked drop (vasoconstriction), whereas those who were unable to live over again the embarrassing experience gave neither a rise nor a fall. The same vasomotor changes are to be expected whether a stammerer actually stammers or whether he lives over again a situation in which he stammered severely; my results showed vasoconstriction (never vasodilatation) in both cases; Fletcher looked for vasodilatation in both and was surprised to find vasoconstriction or else no vasomotor change when the stammerer imagined himself in a situation where he would be apt to stammer. As such a situation would be apt to be more free from arm movements than would a similar attack of stammering, I think that these results of Fletcher's are correct and that the stammering results are misinterpreted.

TABLE 7

Vasomotor changes in normal speakers accompanying shock brought on by short stimuli, long stimuli and compound stimuli

KIND OF STIMULI	NUMBER OF SUBJECTS	NUMBER OF STIMULI	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
			Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
Short	7	67	26	10	1	70	18	2	100	39	7
Long	7	43	43	16	3	196	54	5	300	74	11
Compound	5	7	36	19	10	142	73	19	120	97	28

Table 7 shows the comparative vasomotor changes in shock produced by short stimuli, long stimuli and compound stimuli. All curves are those of seven normal speakers. Short stimuli include those listed in table 3. Long stimuli include reading silently several paragraphs from a very exciting novel or from a very horrible description such as *Murders in the Rue Morgue;* taking an unpleasant, unknown object, such as an artificial snake, out of a cloth bag; seeing material for a

TABLE 8

Vasomotor changes accompanying normal speech

SUBJECT	NUMBER OF PERIODS	AVERAGE LENGTH OF PERIODS	NUMBER WITH NO VASOMOTOR CHANGE	NUMBER WITH UNQUESTIONABLE RISES	NUMBER WITH INITIAL ATTENTION DROP	NUMBER WITH FINAL ATTENTION DROP	INITIAL SHIFT OF ATTENTION						READING AND SPEAKING									FINAL SHIFT OF ATTENTION								
							Fall			t			Rise			t			T			Fall			t			T		
							Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
K	20	69	9	10	2	1	3	2.5	2	22	17	12	11	3.8	2	108	55	25	68	33	17	3	3	3	16	16	16	27	27	27
E	4	57	0	4	0	0	0	0	0				10	7.0	3	60	53	38	48	36	22	0	0	0						
B	5	74	3	1	1	2	4	4.0	4	19	19	19	4	4.0	4	64	64	64	67	67	67	4	3	2	16	13	10	75	64	53
C	7	69	2	3	2	0	2	2.0	2	20	20	20	2	2.0	2	120	77	52	76	66	22	0	0	0						
G	3	63	0	3	0	1	0	0	0				7	5.7	5	43	38	34	60	36	17	7	7	7	23	23	23	45	45	45
L	7	119	2	1	4	3	15	13.0	11	95	37	12	4	4.0	4	110	110	110	100	100	100	19	11	6	12	7	4	44	40	35
M	8	58	3	2	0	3	0	0	0				3	2.5	2	23	18	13	24	22	20	10	6	4	14	10	4	52	35	13
N	3	66	1	0	2	0	4	3.0	2	13	8	5	0	0	0							0	0	0						
Q	5	95	0	0	5	0	7	4.8	3	60	35	15	0	0	0							0	0	0						
9	*62*	75	*20*	*24*	*16*	*10*	6	6.0	4	38	27	14	6	4.2	3	75	55	48	63	41	38	9	6.6	4	16	12	11	49	43	35

bonfire placed and being told to light it at a signal; and watching a mechanical frog set to go off several seconds after being placed. Compound stimuli consist of several short stimuli given simultaneously or in quick succession. It is evident that "t" will be lengthened in long stimuli and in compound stimuli given at intervals of a few seconds and that "T" will be lengthened when the time of maximum fall occurs near the beginning of a long stimulus or after the first part of a compound stimulus. This table shows that the maximum fall, "t" and "T" are greatest for compound stimuli and least for short stimuli. The maximum fall is 60 per cent greater for long stimuli than for short stimuli, and 90 per cent greater for compound stimuli than for short stimuli.

To ascertain whether the vasoconstriction which invariably accompanied stammering was due in part to reading or speaking per se, I had nine normal speakers read aloud and speak under the same conditions as the stammerers.

Table 8 shows the vasomotor changes of these nine normal speakers while they were reading aloud or speaking. These subjects read short stories which were free from excitement and gruesome descriptions so that emotions could not influence the vasomotor changes accompanying reading, and usually read in periods of from one to two minutes in order to give the vasoconstriction which sometimes accompanied the shift of attention to reading, time to eliminate itself half a minute or more before the end of the reading period.

"t" for "reading" was measured from the instant the subject began to read or speak to the moment when the maximum rise occurred on the plethysmogram. "t" for "final shift of attention" was measured from the end of the reading or speaking period to the time when the maximum fall occurred on the plethysmogram. "T" for "reading" included the final attention drop if there was one. The figures in the bottom line of this table denote the averages for their respective columns; the figures in italics, however, denote the totals for their respective columns.

Table 8 shows that there was no vasomotor change in 32 per cent of the reading and speaking periods; that there was an average rise of 4.2 mm. in 39 per cent of these periods; that there was an average attention drop of 6 mm. when the subject began to read or speak in 26 per cent of these periods; and that there was an average attention drop of 6.6 mm. when the subject stopped reading or speaking in 16 per cent of these periods. In addition to the twenty periods in which there was no vasomotor change, there were three periods in which the only vasomotor change was an attention drop at the end of the reading or speaking period; hence the number of periods in which there was no vasomotor change practically equalled those in which there was vasodilatation. It is hard to classify under vasoconstriction, vasodilatation, and no vasomotor change, the periods in which there was an initial attention drop. If the maximum vasoconstriction occurred near the beginning of the period and the needle returned to its normal level as quickly as it did in the final attention drop for the same subject, it seemed logical to classify that period under either vasodilatation or no change; otherwise a period seemed to belong under vasoconstriction. As the needle usually returned to normal more quickly than it did in the final attention drop in the periods classified under vasodilatation and no change, and as it might have risen considerably had it not had to regain what was lost during the initial attention drop, I classified all such periods under vasodilatation. This classification gave 5 vasodilatations and 11 vasoconstrictions, which, added to the 24 unquestionable vasodilatations and the single unquestionable vasoconstriction, gave 71 per cent vasodilatations and 29 per cent vasoconstrictions where there was a vasomotor change. This agrees with the results obtained by Doctor Anderson, who found that mental work was accompanied by vasodilatation in 75 per cent of the periods of mental work, and that this proportion for reading was less than that for most other kinds of mental work. Vasodilatation predominated in some subjects, no vasomotor change in others. Attention drops occurred relatively frequently in some subjects and seldom if at all in others. Vasodilatation followed all of the initial attention drops, but a rise above normal occurred in but two periods; the needle returned to normal at or before the end of the reading or speaking period in about half of the periods. The initial attention drop was but two-fifths of that accompanying stammering and was reached twice as quickly in periods averaging approximately the same length, showing that this vasoconstriction was usually due to the shift of attention rather than to the reading. The maximum rise accompanying normal speech occurred at practically the same instant as the maximum fall occurred during stammering, i.e., 55 versus 53 seconds after the beginning of the period. The time of recovery averaged four-fifths of that in stammering, 41 versus 51 seconds, and was measured from the end of "t," which usually occurred several seconds before the end of the period, and not from the end of the period as in stammering.

It will be seen, therefore, that the vasoconstriction which always accompanied stammering was not caused by reading or speaking per se, but by stammering.

I watched the writing lever of the piston recorder whenever I gave a stimulus and noticed there was a latent period of a few seconds before it began to drop. I ran the kymograph at maximum speed for a few curves with a number of subjects to determine more accurately the length of this latent period. I found that this ranged from 1.6 second to 4.0 seconds, and that it averaged 3.4 seconds; this corresponds very closely with the latent period for smooth muscle. The average varied slightly in different subjects. The latent period was about the same in some subjects whether the stimulus was intense or slight; in other subjects it varied by as much as 2 seconds. The fall always began at the same point whether there was a temporary rise or not, and this rise never amounted to more than 2 mm. unless there was a violent movement of the finger. There was more apt to be a temporary rise when the subject jumped or when the stimulus was very intense. I feel sure this temporary rise was due to movement in the finger for it occurred too soon to be caused by vasodilatation. An unexpected stimulus causes one to tighten up more or less even if one does not actually jump; this tightening up is instantaneous but one does not relax at once. The jump itself, if there is one, does not last more than a fraction of a second and is registered as a vertical line on the curve; but there is a gradual readjustment after the jump which lasts for a few seconds. This lasts longer in the arm than in a single finger and explains the same temporary rise mentioned by so many other experimenters. Being convinced that this rise is due to movement and not to any vasomotor change, I have classed it under the general heading of fall, indicating vasoconstriction.

To determine whether the thought process was most impeded at the time when the stimulus was given or "t" seconds later when vasoconstriction in the periphery was at its maximum, I tied around the wrist of each subject's right hand a cord connected through pulleys with a writing lever which registered directly below the plethysmogram. I ruled three columns about one inch apart on a piece of paper and told the subject to add thirteen to the last number in the left column, then seventeen to the last number in the middle column, then twenty-one to the last number in the right column, then thirteen again, and so on; whenever his arm moved to the next column, it displaced the writing needle vertically. The horizontal lines on the record between two vertical lines measure the number of seconds it took the subject to perform each addition. I gave several short, unexpected stimuli at intervals while the subject was performing this triple addition. A comparison of the plethysmogram with the curve below it showed that the abnormally slow solutions occurred when vasoconstriction was at its maximum, not when the stimulus was given.

SUMMARY

1. Shock and stammering are accompanied in every case by marked vasoconstriction. In a few cases this is preceded by a short, inconspicuous rise in the plethysmogram that is probably caused by arm movement.
2. Vasoconstriction does not begin until about three seconds after the stimulus is given.
3. Long stimuli are accompanied by greater vasoconstriction and slower recovery than are short stimuli.
4. Compound stimuli are accompanied by greater vasoconstriction and slower recovery than are either long or short single stimuli.
5. The more intense the stimulus and the more unexpected the stimulus, the greater is the vasoconstriction, the more rapid the vasoconstriction and the slower the recovery.
6. All the tables show marked individual differences even on the same record.
7. The greater the vasoconstriction, the more is verbal imagery impaired.
8. Those subjects who experience the greatest vasoconstriction during shock and stammering also require the longest time for recovery.
9. Marked peripheral vasoconstriction takes place more rapidly than does slight vasoconstriction.
10. Stammerers experience greater and more rapid vasoconstriction and slower recovery in shock than do normal speakers.
11. Stammerers as a class experience slightly greater vasoconstriction in shock than in stammering. The time of recovery is practically the same in both stammering and in shock. Those subjects in whom the fear of stammering is pronounced, however, experience greater vasoconstriction and slower recovery in stammering than in shock.
12. Vasoconstriction continues throughout the stammering interval; if any vasomotor change accompanies normal speech it is vasodilatation in a large majority of periods.

13. Fear of stammering with no attempt at speaking produces vasoconstriction in the periphery as does actual stammering.

14. Stammerers cannot speak without hesitancy during peripheral vasoconstriction.

15. All stammerers breathe abnormally while stammering. Every stammerer has a characteristic form of breathing while stammering.

THEORETICAL CONCLUSIONS

Stammering and shock are induced emotional disturbances accompanied by the same vasomotor changes. My experiments show that the vasomotor changes in stammerers as a class are of about the same magnitude and in the same direction in both stammering and shock. Unusually intense shock, such as the unexpected report of a pistol, is accompanied by greater vasomotor changes in the periphery than is stammering. Severe stammering, on the other hand, is accompanied in most subjects by greater vasoconstriction in the finger than is shock produced by any stimulus but the pistol. The time of recovery is about the same for stammerers as a class both in stammering and in shock, but is greater for most individuals in stammering than it is in shock produced by stimuli other than the pistol.

The experiments of Mosso and Shepard on trephined subjects reported in an earlier part of this monograph show that shock is always accompanied by vasoconstriction in the periphery and by vasodilatation in the brain. As my results for the periphery confirm those of these experimenters, and as I found approximately the same vasoconstriction in the periphery for stammering as I found for shock, there is every reason to suppose that stammering, like shock, is accompanied by vasodilatation in the brain and hence by cerebral congestion. I have been unable to find a trephined stammerer; as soon as I am able to locate a wounded stammering soldier who has been trephined, I hope to induce him to serve as a subject so that I can prove this supposition that stammering, like shock, is accompanied by cerebral congestion.

It is a well-known fact that fright, which is accompanied by cerebral congestion, may cause temporary partial paralysis of the speech mechanism and of other parts of the body. Persons lose their heads in panics and do inappropriate acts for which they censure themselves later.

Cerebral congestion affects the speech more easily than it affects any other human activity. Many persons have been struck dumb in intense fright. Many normal speakers repeat words or even stammer when greatly excited, and talk incoherently when mentally tired or confused; stammerers experience the greatest difficulty under these same conditions. Several of my normal subjects whose introspection is reliable reported that the words they were about to speak suddenly left their minds when the whistle blew and did not return for several seconds. Some of them still visualized the words and their minds seemed otherwise to work normally, yet the *sounds* of the words left them; hence they were unable to say the words. When vasoconstriction in their fingers ceased, the sound of the words reappeared and they were able to speak them. The same phenomenon occurs in stammering. The fear of stammering and the excessive effort put forth in forcing out difficult words cause cerebral congestion which causes the auditory images of the words the stammerer is about to speak to disappear as he attempts to utter them, just as the whistle banished the sound of the word the normal speaker wished to use and made of him a temporary stammerer. My plethysmograms show that one stammers only when there is vasoconstriction in the periphery and hence, as I have assumed, cerebral congestion. As the stammerer overcomes the impediment in his speech this peripheral vasoconstriction diminishes, and ceases when he is cured.

We see, then, that the fear of stammering and the forcing out of hard words which accompanies stammering cause vasoconstriction in the periphery and cerebral congestion. The latter blurs verbal imagery, especially auditory verbal imagery, and makes it impossible for the stammerer for the time being to recall a part or the whole of the word he wishes to speak at the moment he has to say it. As vowels are governed primarily by auditory imagery they are the stammerers' bugbears. The stammerer prolongs continuous consonants for seconds and repeats closed consonants over and over until able to recall the vowel that follows. The more the vowel is inhibited, the harder he forces out the preceding consonant, the more he thereby increases cerebral congestion, the more impossible it becomes to recall the vowel, and the worse he stammers. Many stammerers are thus led to believe that their trouble lies with the consonants they thus overdo and, by concentrating their mind on trying to force out these consonants, they keep the mind from recalling the vowel which fails to come promptly, and thus increase their stammering.

I have assumed, temporarily, that stammerers have normal verbal imagery when they are not actually stammering. I am conducting at the present time a comparative study of verbal imagery in stammerers and normal speakers which will either confirm or overthrow this assumption. This study will be published in a few months.

My experiments confirm Dr. C. S. Bluemel's theory that stammering is caused by transient auditory amnesia in the auditory speech center brought on by cerebral congestion.

BIBLIOGRAPHY

The following references are mentioned in the earlier pages of this pape For a complete bibliography, consult the bibliographies given in the articles by Angell and Thompson (3), Anderson (1), Shepard (29), Stevens (32), and Weber (35).

(1) ANDERSON: Circulatory reactions during physical and mental work, 1917. (This has not been published, but may be consulted in the Widener Library of Harvard University.)
(2) ANGELL AND McLENNAN: Psychol. Rev., 1896, iii, 371.
(3) ANGELL AND THOMPSON: Psychol. Rev., 1899, vi, 32.
(4) BERGER: Über die Korperlichen Ausserungen psychischer Zustande. Jena, 1904–7. Two volumes with an atlas.
(5) BINET AND COURTIER: L'Annee psych., 1895, ii, 87.
(6) BINET AND COURTIER: L'Annee psych., 1896, iii, 65.
(7) BINET AND COURTIER: L'Annee psych., 1895, ii, 87.
(8) BINET AND COURTIER: L'Annee psych., 1896, iii, 42.
(9) BINET AND COURTIER: L'Annee psych., 1897, iv, 30.
(10) BINET AND HENRI: L'Annee psych., 1895, ii, 141.
(11) BINET AND HENRI: La Fatigue Intellectuelle, Paris, 1898.
(12) BONSER: Psychol. Rev., 1903, x, 120.
(13) DROZYNSKI: Psychol. Stud., 1911, vii, 83.
(14) FERE: Sensation et Mouvement, 1889, 1900.
(15) FRANKFURTHER AND HIRSCHFELDT: Arch. f. Physiol., 1909, 407.
(16) FRANKFURTHER AND HIRSCHFELDT: Arch. f. Physiol., 1912, 215.
(17) GENT: Phil. Stud., 1903, Bd. 18, 715.
(18) GLEY: Etude de psych. physiol. et pathol., 1903.
(19) GLEY: Arch. de Physiol., normale et pathol., 1881.
(20) HALLION AND COMPE: Arch. de Psychol., 1894, 381.
(21) KÜPPERS: Zeitschr. f. Neurol. u. Psychol., 1913, xvi, 517.
(22) LEHMANN: Die Hauptgesetze des menschlichen Gefühlslebens, 1892.
(23) LEHMANN: Die Korperlichen ausserungen psychischer Zustande, Leipsig, 1899–1905. Text and atlas.
(24) LOMBARD AND PILLSBURY: This Journal, 1899, iii, 186.
(25) MACDOUGALL: Psychol. Rev., 1896, iii, 158.
(26) MOSSO: Die Temperatur d. Gehirns, 1894.
(27) MOSSO: Fear, transl. from 5th ed. of the Italian by Lough and Kiesow, 1896, New York,

(28) Mosso: Über den Kreislauf d. Blutes im mench. Gehirn, 1881.
(29) **Shepard**: Amer. Journ. Psychol., 1906, xvii, 522.
(30) **Shepard**: The circulation and sleep, 1914, New York.
(31) **Shields**: Journ. Exper. Med., 1896, i, 71.
(32) **Stevens**: Amer. Journ. Psychol., 1905, xvii, 409.
(33) **Stevens**: Amer. Journ. Psychol., 1903, xiv, 13.
(34) **Weber**: Arch. f. Anat. u. Physiol., Physiol. Abt., 1914, 305.
(35) **Weber**: Der einfluss psychischer Vorgange auf den Korper, Berlin, 1910.
(36) **Weber**: Arch. f. Anat. u. Physiol., Physiol. Abt., 1914, 290.
(37) **Weber**: Arch. f. Physiol., 1910, 367.
(38) **Weber**: Arch. f. Anat. u. Physiol., 1914, 330.
(39) **Weber**: Arch. f. Physiol., 1910, 455.
(40) **Weber**: Arch. f. Anat. u. Physiol., 1914, 385.
(41) **Weber**: Münch. med. Wochenschr, 1910, lvii, 1891.
(42) **Weber**: Zeitschr. f. Physiol., 1910, lvi, 307.
(43) **Weber**: Arch. f. Physiol., 1911, 401.
(44) **Weber**: Arch. f. Physiol., 1913, 205.
(45) **Weld**: Amer. Journ. Psychol., 1912, xxiii, 245.
(46) **Wundt**: Phil. Stud., 1900, xv, 149.
(47) **Wundt**: Physiologische Psychologie, 5th ed., ii, 263.

A complete bibliography of works on stammering will be found at the end of the following references.

(48) **Bluemel**: Stammering and cognate defects of speech, 1913, New York.
(49) **Fletcher**: Amer. Journ. Psychol., 1914, xxv, 201.

EXPLANATION OF FIGURES

Unless otherwise stated, the top line in figures 3 to 12 is the time line, the second line is the stimulus line, the third line is the thoracic pneumogram, and the bottom line is the plethysmogram.

Each notch in the time line in figure 3 represents two seconds. As the kymograph ran at approximately the same speed on all of these curves but those shown on figures 11 and 12, the time between any two points in figures 4 to 10 inclusive can be obtained approximately by measuring the distance between the two points and counting the number of notches in a like distance on figure 3 and multiplying by 2. (Allowance must be made for different reductions in reproduction). The exact time is recorded between two notches on the time line in all records, but all or part of this is sometimes missing from the part reproduced.

A notch on the stimulus line indicates when a stimulus was given or when a subject began or stopped reading or talking.

The top of the pneumogram indicates full lungs and the bottom empty lungs, unless otherwise stated.

The top of the plethysmogram indicates vasodilatation, the bottom vasoconstriction.

The following curves are all typical reactions; I have avoided reproducing extremes or abnormal curves. If any reader wishes to see my other curves, he is invited to examine those in my album at the Boston Stammerers' Institute.

Figure 3 shows a typical reaction of a normal speaker to shock. This curve was made by subject D on February 26, 1918. The tambour that recorded the thoracic breathing was inverted in this record so that the top of the pneumogram represents empty lungs and the bottom full lungs. At the notch marked *22*, I placed a mechanical frog near the subject. At the next notch, about a quarter of an inch to the right, this frog jumped 4 feet into the air without the slightest warning. At *23*, the subject read silently a dull book until a point well beyond the part reproduced; this occupied the subject's attention and thus prevented arm movements. Note the fall of the plethysmogram when the frog was placed, the temporary rise when the frog jumped (due here to movement when the subject jumped), the decided drop following this brief rise, and the much slower return to normal. Other curves show that recovery is the same whether the subject is in a state of reverie or is reading silently. The straight line tangent to the plethysmogram at its two ends is the path that would have been traced had no stimulus been given.

Figure 4 shows two typical reactions of a stammerer to shock. This curve was made by subject Wi on August 1, 1918, a few minutes before the curve reproduced in figure 5. At *W*, a shrill whistle was blown without warning and at *Y*, the operator gave a loud, sudden yell. The time line shows that an interval of a minute and a half elapsed from the notch to the right of *W* to the notch to the left of *Y*. The straight lines on the right hand half of the plethysmogram show how "Fall," "t" and "T" are measured; the line tangent to the plethysmogram at its two ends shows the path that would have been traced had no stimulus been given, the vertical line represents the maximum fall and cuts the stimulus line at a point "t" seconds from the notch where the yell was given and "T" seconds from the point of tangency at the extreme right of the reproduction. Note that the drop for the whistle is much greater than that for the yell.

Figure 5 is a typical stammering curve made by subject Wi a few minutes after the curve shown in figure 4. At the left hand stimulus notch, this stammerer was told that he would be called upon to introduce two persons in fifteen seconds. At the longer notch, about half an inch to the right, he began the introduction, stammering considerably; at the next notch, a quarter of an inch to the right, he finished speaking. The long notch in the time line has no significance. Note that the curve fell rapidly soon after the subject was told that he would be asked to introduce two persons and remained low throughout this period of warning, that it fell nearly as much below this point soon after the subject began to stammer and remained low throughout the stammering interval and for a few seconds after stammering ceased, and that it returned much more gradually to normal. The room was cooling down somewhat, hence the curve fell gradually throughout the record.

Figure 6 is a typical curve of a long period of stammering made by Wi on the same day as that reproduced in figure 5. At the notch below *17*, the subject was told to check a trunk to Portland, Maine, in forty-five seconds. At *18*, he began to speak to a baggage master whom he saw clearly in his imagination and I kept him stammering to the first long notch on the time line by asking him unexpected questions about his trunk. The short notches close together on the time line indicate when he was stammering most severely. The two long notches at the right with *1 m* between them indicate a period of one minute. The straight line below the pneumogram represents the path that would have been traced if the subject had not been disturbed. Note that the curve fell rapidly soon after the stammerer was told he would be called upon to check his trunk in forty-five seconds and remained low throughout both this interval and the stammering which lasted about a hundred seconds. There was a gradual arm movement during a part of the period of recovery.

Figure 7 is a typical curve of a stammerer reading without being told in advance that he is to be called upon to read. It was made by Bo on April 23, 1918. At the notch below *3*, he began to read and at *4* he stopped reading. Note the steady fall throughout the reading period and the steady but more gradual rise to normal, beginning with a slight finger movement at the close of the reading period. There is another gradual finger movement near the end of the period of recovery. The pneumogram is inverted in this record.

Figure 8 is a typical curve made by a cured stammerer reading without hesitancy. It was made by Bo on November 26, 1918, after he had been taught how to read without hesitancy. The subject read without stammering for one minute between the single and double notches on the stimulus line. There is a distinct breathing wave in the plethysmogram (due to movement). There was a gradual movement of the finger at the end of the reading period. The curve rose very slowly throughout the record because the air within the tube was warming up and expanding. The breathing appears very much deeper on this record than on the others because a much more sensitive pneumograph was used; this pneumogram is not inverted. Note that the plethysmogram did not fall as it did in figure 7, when the same subject stammered severely while reading a similar passage from the same book.

Figure 9 is a typical curve of a normal speaker reading, showing an attention drop. At *5*, the subject began to read; at *6*, he stopped reading. The time notches above *5* and *6* indicate that he read for exactly one minute. After the attention drop at the beginning of the reading, there was a slow rise to normal with occasional gradual arm movements. The curve rose gradually throughout this experiment, as the air in the tube was growing warmer.

Figure 10 is a typical curve of a normal speaker reading, showing no attention drop. He read for about one minute, beginning at the left hand notch and stopping at *6*. The curve is as regular as that in reverie preceding and following it. The subject's finger moved slightly while he was reading. The engraver omitted the time line which happened not to be needed.

Figure 11 is a typical shock curve traced when the kymograph was running at maximum speed to show the latent period of vasoconstriction. It was made on November 18, 1918, by the same normal speaker who made the curve reproduced in figure 9. At the extreme left hand notch on the stimulus line, I yelled suddenly. The time notches in the right hand half of the record were made at intervals of ten seconds. Note the slight rise due to arm movement the instant I yelled, the distance from this point to the point where vasoconstriction began, the rapid vasoconstriction and the gradual arm movement at the beginning of the slow rise to normal which is reached at the very right hand end of the reproduction. Reference lines made at the right hand end of all four curves show that the four recording needles were working nearly in a straight line. The breathing appeared more shallow than that in the other records because the less sensitive Verdin pneumograph was used.

Figure 12 contains a number of pneumograms taken at maximum speed. The left hand half of the one at the top is that of a normal speaker reading. The right hand half is a typical pneumogram of a cured stammerer reading without hesitancy. The pneumogram just below is that of a stammerer who habitually spoke on empty lungs. The middle one is that of a stammerer who habitually spoke on full lungs. The two near the bottom are those of stammerers who spoke now on full lungs and now on empty lungs; note their irregularity.

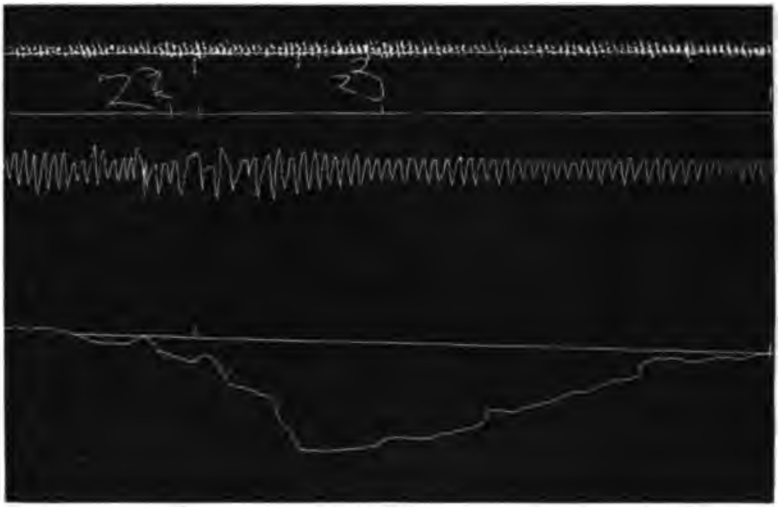

Fig. 3. Three-fourths natural size

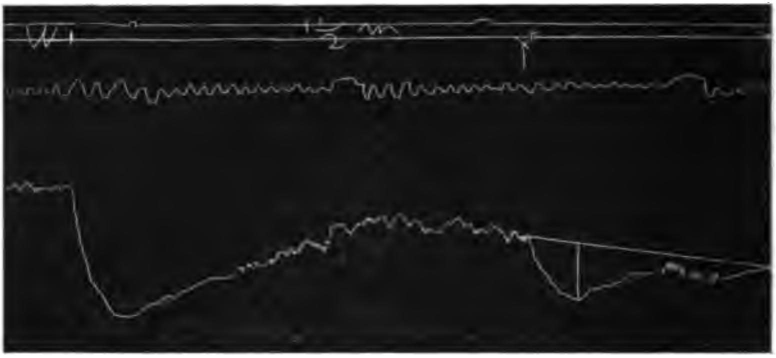

Fig. 4. Three-fifths natural size

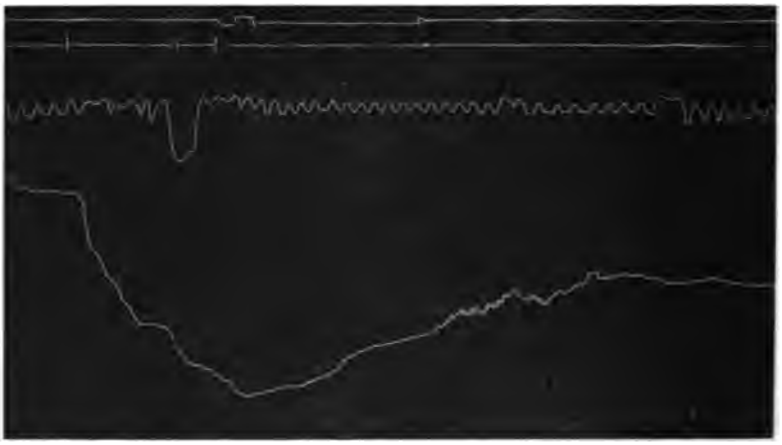

Fig. 5. Two-thirds natural size

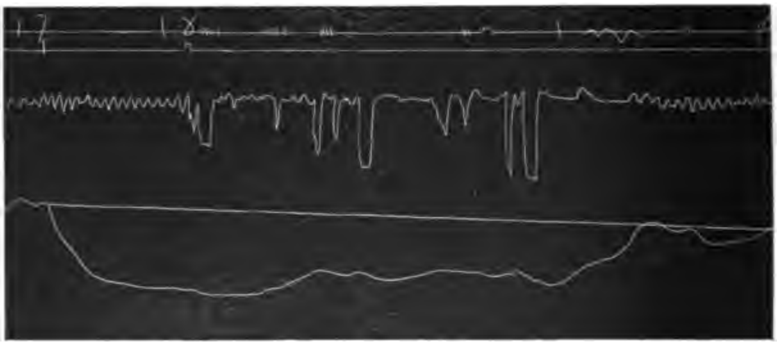

Fig. 6. Three-fifths natural size

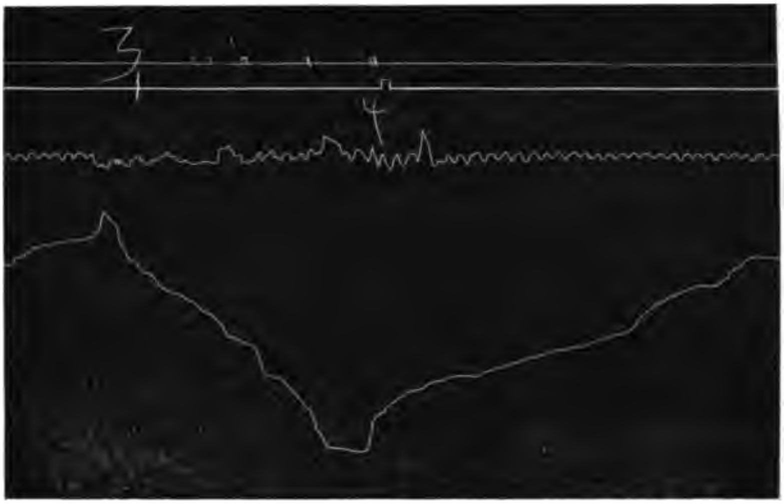

Fig. 7. Four-fifths natural size

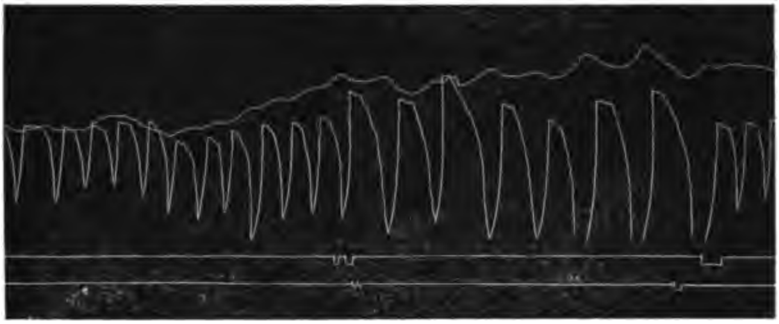

Fig. 8. Three-fifths natural size

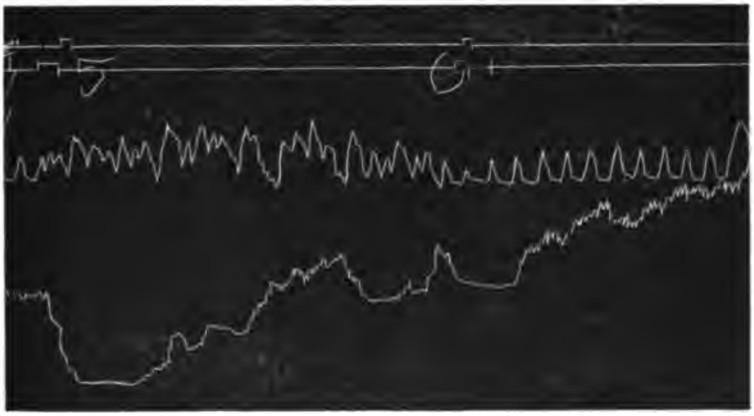

Fig. 9. Three-fourths natural size

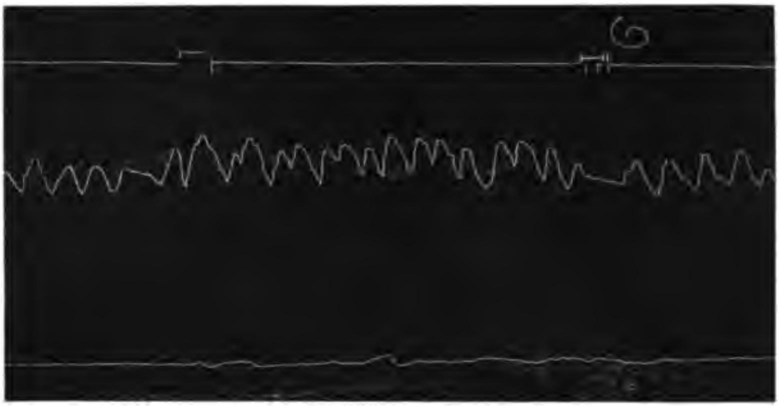

Fig. 10. Three-fourths natural size

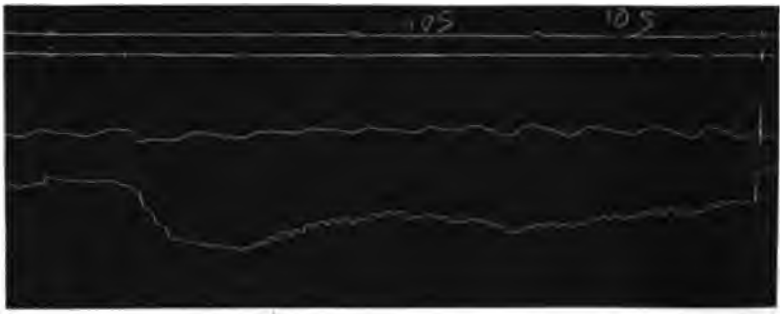

Fig. 11. One-half natural size

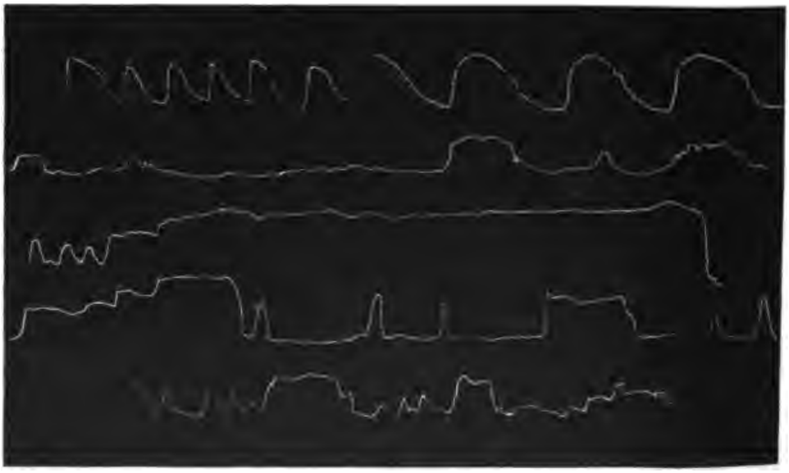

Fig. 12. One-third natural size

A NOTE ON INTRAVASCULAR FAT IN RELATION TO THE EXPERIMENTAL STUDY OF FAT EMBOLISM IN "SHELL SHOCK"

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The prominence recently given to the hypothesis that "explosive shock" is due to fat embolism has led to the histological examination for intravascular fat in the brains and other organs of dogs subjected to concussion by Dr. D. R. Hooker, for the Committee of Physiology of the National Research Council, in his studies on the physiological effects of air concussion. These observations have been controlled by the examination for fat in the blood vessels of normal laboratory animals.

METHODS

Material preserved in formaldehyde and fresh tissues have been used in this study. Free-hand sections made with a razor blade or frozen sections, pieces of the meninges with their vessels and pieces of the choroid plexuses of the brain have been stained with Sudan III, Scharlach R and with osmic acid. Staining with Sudan III has been employed most frequently and as follows:

Stain 1 to 3 minutes in
  Sudan III, saturated solution in 70 per cent alcohol........... 100 cc.
  Sodium hydroxide (sticks).. 1 gm.
    (filter after sodium hydroxide has dissolved)
Rinse in 60 per cent alcohol.
Pass directly to glycerine.

Note: Sudan III (Grübler) and Aniline Red, Sudan III oil as sold by Eimer & Amend, have been used.

After staining and when cleared in glycerine, all sections or pieces of tissue were examined with a binocular microscope with a 32 mm. objective and low-power oculars. For closer observation, some preparations were mounted on slides under cover glasses in glycerine and examined with the compound microscope.

In order to demonstrate accurately the intravascular position of fat droplets in the choroid plexuses and in other small cerebral vessels with collapsed walls, India ink has been injected through the arteries of the head prior to fixation. Preparations of the choroid plexuses stained with Sudan III, after this injection with India ink and fixation with formaldehyde, show clearly the position of the fat droplets with reference to the ink particles contained in the vessels (see dog 13).

To test the accuracy of the staining with Sudan III, osmic acid has been used. Sections or pieces of tissue containing vessels holding droplets stained with Sudan III have been treated with 1 per cent osmic acid while under observation with the microscope. The characteristic blackening of the droplets begins at once, replacing or superimposed upon the clear orange color which follows treatment with Sudan III.

Examination of the liver, dura and choroid plexus, fresh, without previous fixation (dog 12), and observations on the vascular content of the liver in dog 13 have been made to control the observations on material fixed in formaldehyde.

Dogs. Observations on intravascular fat have been made on ten dogs, five control animals, four exposed to and shocked by air concussion due to gun-fire and one subjected to prolonged anesthesia. The histories and findings in these dogs are as follows:

Dog 1. Adult male. Subjected to air concussion and shocked. Dead ten and a half hours after exposure and immediately preserved with formaldehyde. Central nervous system saved.

Vessels of the dura and arachnoid and those of the choroid plexus of the third ventricle show intravascular fat.

Dog 2. Adult male. Subjected to air concussion and shocked. Dead four hours after exposure and immediately injected with formaldehyde. Central nervous system saved.

Fat droplets present in vessels of the choroid plexus of the third and lateral ventricles and in vessels of the dura.

Dog 7. Adult male. Subjected to air concussion and shocked. Dead two hours after exposure. Central nervous system fixed by injection with formaldehyde.

Fat droplets present in vessels of the choroid plexus of the fourth and lateral ventricles and in the venous capillary expansions of the dura.

Dog. 10. Adult male. Following morphia and the destruction of ear drums and ossicles under ether, subjected to air concussion with abdomen covered with cotton pad and plaster bandage. Shocked. Dead thirty minutes after exposure. Central nervous system and viscera preserved in formaldehyde.

Fat droplets present in the vessels of the choroid plexus of the fourth ventricle and in vessels of the dura.

Smaller vessels of the lungs and kidneys contain occasional droplets. No fat found in vessels of the spleen, liver, pancreas, cardiac muscle or suprarenal.

Dog A. Adult male. One hour after the administration of one grain of morphia, ether given and continued through tracheal cannula for two and a half hours, when the animal was bled to death. Blocks of the brain were removed fresh and the remainder of the central nervous system preserved immediately in formaldehyde.

Fat droplets present in the vessels of the choroid plexus of the third and fourth ventricles and in vessels of the dura and corpus striatum.

Dog B. Adult male (control for A). Killed by bleeding following local anesthetic. Central nervous system fixed in formaldehyde.

Vessels of the dura and of the choroid plexus of the third and lateral ventricles contain fat droplets.

Dog 9. Adult male (control for no. 7). Killed by intravenous injection of ether and immediately injected with formaldehyde. Central nervous system saved.

Droplets of fat found in vessels of all of the choroid plexuses, the dura and arachnoid and in vessels within the substance of the corpus striatum.

Dog 11. Young adult male (control). Killed with illuminating gas. Central nervous system and viscera immediately fixed by immersion in formaldehyde.

Fat droplets present in all choroid plexuses, in vessels of the dura and arachnoid and in the posterior inferior cerebellar artery.

Vessels of the lungs, kidney and liver contain fat droplets. None demonstrated in vessels of the spleen, pancreas, suprarenal or cardiac muscle.

Dog 12. Adult male (control). Killed with illuminating gas. Central nervous system and viscera fixed by immersion in formaldehyde, except a portion of the liver, a piece of dura and a part of the choroid plexus of the fourth ventricle, which were examined fresh.

When examined without previous fixation, vessels of the liver, of the dura and of the choroid plexus of the fourth ventricle contained fat droplets stainable with Sudan III and with osmic acid.

After fixation with formaldehyde, fat was demonstrated in vessels of the dura, arachnoid and all of the choroid plexuses.

After fixation with formaldehyde, fat droplets were observed in vessels of the lung, liver, kidney and suprarenal. None was seen in vessels of the spleen or cardiac muscle.

Dog 13. Adult female (control). Killed with illuminating gas. Liver removed entire with blood contained and immersed in formaldehyde. Both common carotid arteries isolated and warm Ringer's solution passed through the head until the solution ran clear. Tourniquet applied to neck below the site of carotid exposure. Solution of India ink injected in the right carotid until it ran from the left carotid. Left carotid then clamped and a little more ink injected with syringe into the right carotid. Both carotids then tied, brain exposed and head fixed by immersion in formaldehyde. The vessels of the choroid plexuses and of the meninges and brain were incompletely injected with the ink.

Fat droplets demonstrated, after staining with Sudan III, in vessels of the dura, arachnoid and in vessels of all choroid plexuses.

In the brain of this dog, following injection with India ink and staining with Sudan III, particles of ink and fat droplets were sometimes found together in the blood vessels. Many of the capillary loops of the choroid plexuses, incompletely injected with the ink, were found to be occupied by fat droplets. The capillary loops of the choroid plexuses, well injected with the ink, were usually found free from fat droplets. In the flat venous capillary expansions found in the dura, particles of ink and fat droplets, more or less finely divided, were found constantly together.

Pieces from the surface of the liver, immersed in formaldehyde as above, were taken 3, 5, 24 and 72 hours after the beginning of fixation and examined for fat. Intravascular droplets were demonstrated in all. At the end of 24 and 72 hours, after the beginning of fixation, pieces of liver were taken from the surface and from 5 and 10 mm. beneath the surface and examined for fat. All pieces showed intravascular fat droplets.

In the routine examination of brains foı intravascular fat the choroid plexuses and dura were chosen as parts containing a great number of capillary vessels. They proved to be easily handled by the methods employed and easily studied when spread and observed with a binocular microscope. Sections of the brain itself were made in some cases but the difficulty of handling these sections and clearing them in glycerine for accurate observation, the necessity of thinness and the relatively small number of capillaries available for study in even a considerable number, made their use impractical. The choroid plexuses were removed and stained entire and when spread in glycerine after clearing, presented a great number of capillary vessels covered only by the transparent ependyma. In several cases fat droplets were found in all the choroid plexuses taken from a single brain but usually such droplets were confined almost wholly to one plexus. The choroid plexus of the third ventricle, in our observations on dogs, has held by far the greatest number of fat droplets; in several cases almost a whole half of the plexus contained droplets, practically every one of the many capillary loops being filled with them.

Pieces of the dura were found to be quite easily handled and cleared. When cleared and examined with the high power objective of a compound miscroscope, many of the capillaries and the capillary expansions between the arterial and venous capillaries were found to contain blood and, with the blood, droplets of fat. Injection with formaldehyde or Ringer's solution frees the larger dural vessels of blood but the contents of most of the capillaries and their expansions seem to be incompletely removed by such injection.

In the dog, as well as in the rabbit and cat, intravascular fat has been found in the form of single droplets or in groups of several droplets. These droplets vary in size from those easily seen with the binocular microscope to those just visible with the low-power objective of the compound microscope. In the cerebral vessels single droplets only have been observed occurring singly in the larger vessels and often in rows in the capillary loops of the choroid plexuses. In the choroid plexuses large droplets have been quite constantly found, droplets whose diameter is usually greater, when observed after the death of the animal, than that of the capillary containing them. In the venous capillary expansions of the dura, on the other hand, most of the droplets observed have been small, little larger than the red blood corpuscles occurring in the vessels with them. There is evidence in some preparations of the dura that several of these small droplets may coalesce to form larger ones. In sections of the lung, fat droplets have been frequently found pressed out in passing through a small capillary loop, so that they present after fixation a bent cylindrical form completely filling the small capillary.

Careful examination of the above findings in dogs shows that fat has been demonstrated in the blood vessels of all examined, no matter whether the individual has been shocked by air concussion or not. No quantitative or qualitative differences have been observed between the fat found in the vessels of the shocked animals and the intravascular fat of normal controls. Exact quantitative estimation of fat present in the vessels is of course impossible with the methods used, but its incidence in various parts of the vascular system subjected to examination in the routine study of ten dogs would indicate roughly a considerable quantity in all. If there is any difference between the amount present in the vessels of shocked and control animals, the above observations would lead one to believe that there is more in the control dogs than in those shocked.

It is interesting to note also that the fat present in the vessels examined does not seem to have been affected by the cause of death; animals dying from shock, those killed by bleeding, with ether or illuminating gas, showed no essential differences in the fat present. Death by bleeding certainly would remove most of the fat contained in the vessels completely emptied, but it is evident that death by bleeding does not remove the fat droplets contained in the small vessels examined. Further, in dog 13, where the intravascular position of the droplets contained in the capillary vessels of the choroid plexuses and in the capillary venous expansions of the dura was established with certainty, the common carotids were cut, the animal allowed to bleed, the branches of the common carotid then being washed clean with warm Ringer's solution before the injection of India ink. Notwithstanding this bleeding and washing, fat droplets were still found along with the injected ink particles It is evident then that bleeding or washing out the vessels of the head after bleeding fails to remove completely the fat droplets found in the capillary vessels examined. In the animals injected with formaldehyde for purposes of fixation, it follows that the fat droplets under observation have been little if at all affected by the injection.

It might be considered possible that the fat droplets observed in the vessels after fixation with formaldehyde were changed in size, aggregation and staining reaction by post-mortem factors and fixation. To control such possibilities, sections of the liver and pieces of the dura and choroid plexus of the fourth ventricle were stained with Sudan III and osmic acid, fresh, as soon after the death of the animal as they could be removed (see dog 12). Fat droplets, similar in size, location and staining reaction to those found in the same animal after fixation with formaldehyde were observed in this fresh tissue. Further, that the length of time of fixation with formaldehyde or its penetration, within the limits of our observations, do not change the amount or character of the fat recognizable histologically in vessels of the liver, has been shown by the observations on dog 13 above. It seems probable then that fixation in formaldehyde has not altered the frequency or character of the fat droplets demonstrated histologically in the present observations.

Rabbits. Four large normal rabbits, bled to death for the production of normal serum, were used in these observations. These rabbits averaged about 2,500 gm. in weight and as blood was withdrawn, Locke's solution was supplied intravenously, each rabbit yielding approximately 300 cc. of blood after receiving about 150 cc. of Locke's solution. During this procedure the blood vessels of these rabbits must have been well irrigated so that the amount of intravascular fat found may not represent what was present before the bleeding. It seems probable, however, from our findings in the brain of dog 13 following bleeding and irrigation with warm Ringer's solution, that the amount of fat in the cerebral vessels examined in these rabbits was little affected. The amount present in the other organs examined may have been reduced. The brains and lungs of all four of these rabbits were preserved in formaldehyde. Other viscera from one rabbit were also saved in formaldehyde.

Fat droplets were found in the cerebral vessels of all four rabbits, particularly in the dural venous capillary expansions and in the vessels of the choroid plexuses. Thick sections were made from blocks taken at random, one from each pair of lungs. Fat was demonstrated in the capillary vessels in two of the four. In the other viscera saved from one rabbit, the kidney alone showed intravascular fat after a routine examination of two sections from each organ. These fat droplets were observed in the cortical vessels of the kidney.

Cats. The cats first examined, series 1, had been used for other experiments in this laboratory. Some died or were sacrificed with acute meningeal infection or following intravenous and subarachnoid injections of various sorts. These cats had been fed on a comparatively fat-free diet and following the experiments to which they were subjected were not, at the time of death, in a state of good nutrition. It will be seen from series 1 below, that intravascular fat was found in none of the choroid plexuses taken from such animals and that the capillary vessels of only five of sixteen pairs of lungs examined were shown to contain fat.

Series 1. Cats fed for some time on a comparatively fat-free diet, some with acute meningeal infection, others subjected to intravenous and subarachnoid injections.

Choroid plexuses of brain. Of ten examined, none showed intravascular fat droplets.

Lung (one block from each pair of lungs). Of sixteen examined, five showed a few fat droplets in capillary vessels (these droplets fewer and more scattered than those observed in pulmonary vessels of the dog or rabbit).

In view of the above, an attempt was made to feed cats a diet rich in fat, preparatory to examination of intravascular fat. The first series of two kittens was complicated by the appearance of distemper which shortened the period of feeding, but in spite of this the kittens showed relatively more intravascular fat than was observed in the cats in series 1.

Series 2. Two kittens fed cream for three days; both contracted distemper, one died in cage. At autopsy, evidence of meningitis in both.

Choroid plexuses of brain. Three droplets found at tip of one of the capillary loops in the choroid plexus of the third ventricle in one kitten.

Dura. Finely divided fat droplets present in the smaller veins and venous capillary expansions in both.

Lung (one block from each pair of lungs). Several groups of free intravascular fat droplets were present, in addition to many intracellular granules.

Kidney (one block from one kidney of each kitten). Fat droplets present in vessels of the cortex in addition to intracellular fat of the cortical tubules in both kittens.

Finally, three healthy, well-nourished, normal adult cats were taken and fed a diet rich in fat for five days before killing. A fourth, normal healthy male, was killed as received without feeding and included in this series. While the amount of fat demonstrated histologically in the blood vessels of these cats seems relatively less than that found in dogs and rabbits, still it is greater than that observed in the poorly nourished cats of series 1. Further, in this series thirteen organs were examined from each of two fat-fed cats and one normal adult cat killed without feeding. In one fat-fed cat, intravascular fat droplets were found in eight of these thirteen organs and in the other fat-fed cat, in six. In thirteen organs taken from the cat killed without feeding, fat droplets were demonstrated in only four.

Series 3. Normal adult cats—three which were fed a rich fat diet for five days, the fourth, a healthy normal male, killed as received without feeding.

Dura. Fat droplets present in dural vessels of the three fat-fed cats; dura of the fourth not examined.

Choroid plexuses of brain. A single droplet observed in vessels of the choroid plexus of two of the fat-fed cats.

Arachnoidal vessels. Fat droplets present in arachnoidal vessels in one fat-fed cat and in the fourth, killed without feeding.

Lung (one block from each pair of lungs). Fat droplets found in pulmonary vessels of the cat killed without feeding—lungs from three cats examined.

Kidney (three cats, two of which were fat-fed). Fat droplets present in cortical vessels of all kidneys examined.

Sections from eight other organs examined in three cats.

The above observations may indicate that the feeding of a rich fat diet in cats may increase the number of intravascular fat droplets, recognizable histologically.

Of the three fat-fed cats in series 3, two were killed with illuminating gas, the third with ether. No essential differences in the incidence or amount of the intravascular fat was observed depending on the means of death.

All the observations on cats seem to show that there is present in this animal relatively less intravascular fat, recognizable histologically in the organs examined, than is found in the dog or rabbit. This is most noticeable in the choroid plexuses where in the dog and rabbit, so far as our observations go, it seems to occur constantly in some part of the choroid plexus.

SUMMARY

1. Free fat droplets, stainable with Sudan III, Scharlach R or osmic acid, are found in the blood vessels of the brain, lungs and other organs of the dog and rabbit in considerable number. In the cat, although present, this intravascular fat seems to occur in smaller amount than in the dog or rabbit.
2. Observations on dogs indicate that there are no quantitative or qualitative differences between the fat, recognized histologically, in the vessels of normal control animals and that demonstrated in dogs subjected to concussion or prolonged anesthesia.
3. In cats it seems probable that a rich fat diet increases the incidence and amount of intravascular fat demonstrated histologically.

ON THE ACTION OF CERTAIN SUBSTANCES ON OXYGEN CONSUMPTION

II. Action of Potassium Cyanide on Planaria

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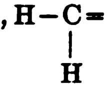
INTRODUCTION AND PRESENTATION OF LITERATURE

In the first paper of this series (1) I believed that I had shown in a sufficiently convincing manner that potassium cyanide decreases oxygen consumption. Sponges were selected as the most favorable organisms for such experiments since in such simple animals the action of drugs like cyanide must be a purely cellular one. As the results were definite and striking and in complete accord with the prevailing opinion regarding the mode of action of cyanides on protoplasm, it did not occur to me that any further experiments with this particular substance were necessary. Subsequently, however, Lund (2) published a paper in which he stated that he was unable to find any decrease in the rate of oxygen consumption in Paramecium in the presence of cyanide. In this paper, the considerable number of investigations now at hand regarding the physiological and pharmacological action of the cyanides were very inadequately considered and no explanation was given as to why Paramecium should differ from other organisms and cells in this respect. The fact is that all investigations, except those of Lund, in which the action of cyanides upon the rate of oxygen consumption has been directly measured have demonstrated a striking decrease in rate of oxidation. In view of this evidence, the only conclusion which is warranted from Lund's experiments is either that Paramecium is an exception which requires special explanation or that the experimental method employed by him is somewhere at fault.

Since, however, Lund seems to think that these experiments entitle him to raise objections to the conclusions which have been drawn by Child (3) and other members of this laboratory from researches in which potassium cyanide (but also other reagents) has been employed, it has become necessary for us to determine directly the action of potassium cyanide upon the oxygen consumption of *Planaria dorotocephala*, the animal which has been extensively used in the experiments referred to. I therefore undertook to measure the rate of oxygen consumption of Planaria in normal and cyanide-containing water. The results of this investigation are reported in the present paper. I may say at once that in my experiments, numbering nearly seventy-five, with concentrations of potassium cyanide ranging from 1/1000 to 1/200,000 mol., I found without exception a decrease in the rate of oxygen consumption in the presence of cyanide. In the stronger concentrations, the amount of oxygen consumed fell to 10 to 20 per cent of the normal value; with increasing dilution of the cyanide solution, the amount of decrease gradually diminishes. In every case complete recovery occurred, the oxygen consumption returning to the former rate when the animals were replaced in water and thoroughly washed.

Before presenting my experiments in detail, I wish to review the literature of this matter since it has been largely ignored by those who would like to think that cyanide does not decrease the oxygen consumption of protoplasm. This literature may conveniently be considered under four heads: the chemical structure and properties of the cyanides; their effect upon enzyme and other catalytic actions; their physiological effects; and their specific action upon the respiration of living material.

The structure of potassium cyanide was elucidated through the work of the late Prof. J. U. Nef. As is well known, Nef believed that many organic reactions involve first the dissociation of the reacting substances into radicals containing unsaturated carbon atoms, and then the union of such radicals with other groups by way of these unsaturated atoms. Starting from the fact that only those organic compounds which contain unsaturated carbon atoms ("double" and "triple" bonded carbon) are markedly reactive, Nef concluded that organic reactions in general are due to the presence of such unsaturated atoms, expecially bivalent carbon. Methylene,  $\mathrm{H}-\underset{\mathrm{H}}{\underset{|}{\mathrm{C}}}=$, is the simplest compound containing bivalent carbon, but this cannot exist, according to Nef, because its bivalent carbon atom is so extraordinarily reactive that it promptly unites with another molecule of itself or with any other available group. Nef suspected that the inorganic and organic cyanides (nitrils) are really substitution products of methylene, having the general formula, $R-N=C=$, and that therefore they must react in a manner identical with the olefines, substances known to contain unsaturated carbon atoms and chemically characterized by their ability to form additive compounds. He found (4) that this was indeed the case, and the cyanides will form additive compounds with great avidity with halogens, halogen acids, sulphur, hydrogen sulphide, phosgene, ammonia, acetyl and benzoyl chlorides, alcohols, aldehydes, primary amines, etc., and polymerize readily. They also add oxygen with great ease, forming $R-N=C=O$ (this may go over into more complex oxygenated compounds). In short, the cyanides[1] are extremely reactive substances; they rank as the most powerful reducing agents known; they interfere with oxidations and unite readily with other groups, particularly unsaturated ones (5), wherever there is an opportunity for them to do so. It should be noted that such unions commonly interfere with the course of the reaction, when cyanide is added to reacting substances, as appears to be always the case in living materials, but they need not necessarily do so since the compound so formed may conceivably be more favorable for the reaction than the original compounds, through greater solubility, for instance.

The reducing power of the cyanides can be readily demonstrated. If potassium cyanide is added to a solution of potassium permanganate, the purple color disappears, a reduction of the septivalent Mn ion to the bivalent condition; and similarly potassium bichromate turns green, hexavalent Cr. having been reduced to trivalent Cr. This capacity of the cyanides for taking up positive charges enables them to keep substances in contact with them in a reduced condition, or conversely, to prevent them from undergoing oxidation, since oxidation consists in the addition of positive charges. Geppert (6), one of the early workers on the action of cyanide on animals, attributes its power to decrease oxygen consumption to this property, and cites several well known oxidations with which potassium cyanide interferes. One of the most striking examples of the power of cyanides to prevent oxidation reactions is that discovered by Mathews and Walker (7), who found that the spontaneous oxidation of cysteine to cystine is retarded by small amounts of potassium cyanide and other isocyanides (nitrils) and prevented by larger amounts. The authors explain the inhibition as due to the union of cyanide with cysteine at the point where oxidation would otherwise occur; this explanation is in accord with the properties of the cyanides as already explained. Curtman and Kaufman (8) have recently called attention to the interference of potassium cyanide with the whole group of oxidations commonly used in quantitative analysis in which iodine is set free through the oxidation of an iodide. Whatever the oxidizing agent employed, some of the liberated cyanide promptly disappears when cyanide is present. Anyone can readily observe for himself the fading of the iodine color in such solutions upon the addition of iodine. Since the Winkler method for the determination of the oxygen content of water depends upon such an oxidation of iodide to iodine, it is quite obvious that it is chemically impossible to determine absolutely the oxygen content of cyanide-containing water by this method. One has to depend upon relative results, or remove the cyanide.

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[1] As Nef points out, substances containing the $N=C=$ radical should properly be designated as isocyanides or isonitrils, since there is a group of substances containing the $C\equiv N$ radical for which the name cyanide or nitril should be retained. In fact, these substances are tautomeric, a solution of any of them containing both radicals, although there is very little $C\equiv N$ present in solutions of potassium cyanide. However, as this terminology has not come into general usage, it is not employed here.

The foregoing examples suffice to demonstrate the inhibiting effect of cyanides upon oxidative reactions *in vitro*, and there is no reason to assume that their action *in vivo* would not be of the same character.

Cyanides also interfere with the activity of organized and unorganized catalyzers. They greatly retard and even completely prevent the catalytic splitting of hydrogen peroxide by finely divided platinum, silver and other heavy metals. This catalysis is commonly believed to be due to the increased adsorptive surface provided by such finely divided metals. This explanation appears to me to be inadequate, for the following reasons. Loevenhart (9) and Kastle and Loevenhart (10) found that cyanide and a number of other substances which retard this catalysis produce their effect by forming a film of insoluble salt around the metal particles. If the effect of the metal particles is solely physical, why should the presence of this film retard the action? On the other hand, the same substances which inhibit catalysis by platinum and silver markedly accelerate the same catalysis when copper and iron are used and the cause of this acceleration is the union of the accelerating substances with the metals to form soluble salts. If the catalysis is due to adsorption on the metal particles, the change of these metal particles into soluble salts passing into true solution ought to interfere with the catalysis, but as a matter of fact it markedly accelerates it. The catalysis therefore is probably primarily a chemical reaction, whose speed depends on the solubility of the substances present. The mechanism of many familiar reactions in which catalyzers are necessary is now known to be chemical; for instance, the formation of esters from fatty acids and alcohols proceeds very slowly unless acid is present. The acid produces its accelerating effect by uniting with the substances present to form a complex salt which decomposes to yield the ester and the original amount of acid very much faster than the ester would be formed in the absence of acid.

Loevenhart (9) and Kastle and Loevenhart (11) state that all substances which catalyze hydrogen peroxide also bring about oxidations, such as the bluing of tincture of guaiacum, oxidation of phenolphthalin to phenolphthalein, etc. Potassium cyanide and other substances which inhibit the catalysis of hydrogen peroxide by certain metals also inhibit these oxidations; but with other metals, such as copper, cyanide causes an acceleration of both the catalysis and the oxidation reactions. The same authors found that, although hydrogen peroxide oxidizes guaiacum to only a slight extent, other peroxides like benzoyl peroxide, lead peroxide, silver oxide, manganese dioxide, readily accomplish this oxidation. Now, the bluing of guaiacum by certain of these peroxides, the first three mentioned, is markedly retarded by cyanide; but in the case of manganese, the oxidation is accelerated.

The conclusion which I wish to draw from these facts is, I think, a significant one,—namely, that the action of cyanide or any other substance upon a given chemical process depends primarily on the nature of the compound formed by the cyanide with the reacting substances. If this compound is less active than the other reacting substances, through insolubility, slight dissociating power or other causes, the reaction will be retarded or even completely inhibited. If the compound formed is more active, then the reaction will be accelerated. This point of view should replace the old dogmatic idea that cyanide is "a specific inhibitor" of oxidations or any other reactions. *It merely happens* that in the case of living, organized materials, the compounds formed with cyanide are nearly always much less reactive than the original substances, so that biochemical processes are in general retarded.

The retarding effect of the cyanides upon certain organized catalyzers, that is, enzymes, has long been known. According to Fiechter (12), the enzymatic power of intact bacteria, yeasts and fungi is completely inhibited by hydrocyanic acid. This author and also Schönbein (13) found that yeast is entirely unable to split glucose in the presence of HNC; the effect is completely reversible, the fermentation proceeding as soon as the hydrocyanic acid evaporates. These retarding effects of the cyanides upon enzyme-containing cells are probably to be regarded as due to inhibition of the general metabolic processes in such cells rather than as actions upon the enzymes themselves.

It is significant, in view of the strong reducing power of the cyanides, that they particularly inhibit the activities of those enzymes associated with oxidation processes in cells; that is, the catalases, oxidases, peroxidases and reductases. Schönbein (13) discovered that practically all tissues, both plant and animal, have the property of splitting hydrogen peroxide into water and oxygen. Loew (14) showed that this property was due to a specific enzyme (or enzymes) to which he gave the name catalase. The recent studies of Burge (15) have shown that in animals catalase is closely associated with oxidation processes, since factors which are known to increase the respiratory exchange, as muscular activity, feeding of thyroid, etc., also increase the catalase content of organs, while factors which decrease respiration likewise decrease the catalase content. The same has been demonstrated for plants by Appleman (16). He found that various treatments which increase the respiratory exchange in potato tubers, such as soaking in ethyl bromide, changing from a lower to a higher temperature, greening in light, sprouting, also augment the catalase content of tubers. The activity of catalase, whether in its normal relations in intact cells, such as yeast cells and red blood corpuscles, or in extracts, is completely inhibited by cyanides, so, that not a trace of oxygen is evolved from the solutions. The effect is quite reversible, the usual amount of oxygen being released when the cyanide volatilizes. This action of cyanide on catalase was discovered by Schönbein (13) and has been repeatedly confirmed since by Schär (17) and Jacobson (18), for example, who used not only hydrocyanic acid but also some substituted cyanides with the same result. Moore and Willaman (19) investigated the action of HNC on the oxidases and catalases of plants. The oxidases from dried tomato leaves or tomato juice are reversibly inhibited by hydrocyanic acid; and oxidases obtained from plants after fumigation with HNC are less active than those from normal plants. Fumigated plants carry on less photosynthesis than normal ones and they fail to use the starch which they produce; this last effect was shown to be due to an inhibition of the consumption and oxidation of sugar, since the basal part of a branch of which the tip alone is fumigated retains its starch. These authors also found that the catalases of plants are markedly inhibited by cyanide. Kastle and Loevenhart (11) worked with plant oxidases which turn tincture of guaiacum blue, and found that such oxidases are inhibited by cyanide. Schönbein and Schär observed (17) that the power of plant tissues to reduce nitrates to nitrites is greatly interfered with by cyanides. Schafer (20) extracted from the bodies of insects oxidases which turn guaiacum blue and give the indophenol reaction, reductases which reduce methylene blue, and catalases. Hydrocyanic acid was found to retard the activity of all three classes of enzymes reversibly. Of particular interest are the observations of Raubitschek (21) and Schultze (22) on the indophenol reaction. When a mixture of dimethyl-*p*-phenylenediamine and α-naphthol is applied to cells or tissues a deep blue precipitate of indophenol results in certain regions. As the reaction is an oxidation it occurs only where oxidations are taking place and has therefore been used to demonstrate the location of oxidases in cells. The authors mentioned have demonstrated that the indophenol reaction does not occur when cyanide is present. Sections made by the freezing method of organs of animals killed by narcotics, asphyxia, carbon monoxide gas and various other means all show the indophenol reaction but sections of organs from animals killed by potassium cyanide (placed in the mouth) give no trace of the reaction. The result is due to the inhibition of oxidases by the cyanide.

The cyanides therefore have a markedly depressing and inhibiting effect upon those groups of enzymes which are generally believed to bring about the oxidation processes in organisms.

As is to be expected, the cyanides have less or no effect upon that class of enzymes whose mode of action is hydrolytic. The observations of various investigators do not, however, agree very well. According to Fiechter (12), even very concentrated solutions of HNC have no effect upon the digestion of fibrin by pepsin, although a 1:2500 solution markedly retards the digestion of egg white by pepsin; a 1:200 solution was required to produce any retardation of the activity of salivary and pancreatic diastase. Fiechter was of the opinion that in concentrations of this strength the acidity was probably the chief factor. Butkewitsch (23) reported that trypsin from the pancreas is very resistant to the action of HNC and that certain proteolytic enzymes obtainable from the seeds of many plants are not only not inhibited by cyanide, but are accelerated. He thought that the first stages of proteolytic splitting were accelerated while the later stages were retarded, but also suggested that the result might be due to the acidity of the hydrocyanic acid. Similarly Vines (24) found that 1 per cent HNC has no effect upon the activity of the proteolytic enzyme found in the pitchers of the pitcher plant, although the activity of glycerin extracts of the walls of the pitcher was slightly retarded by cyanide. The enzyme papain, a protease from the papaw, is remarkably accelerated by HNC, according to Mendel and Blood (25) and Frankel (26), the effect being due to the NC radical and not to the hydrogen ion. In contrast to these results are those of several investigators who report an inhibiting effect of cyanide upon similar enzymes. Abderhalden, Cammerer and Pincussohn (27), working with an enzyme from yeast which splits polypeptids, found that its activity was accelerated by very low concentrations of KNC (about 1/30,000 to 1/150,000 per cent), first accelerated and then retarded by less dilute solutions, and greatly retarded by stronger solutions. Chittenden and Allen (28) found that KNC prevents the digestion of fibrin by pepsin (a result exactly contrary to that of Fiechter), and retards the action of trypsin and ptyalin. Mercuric cyanide had similar effects. Some enzymes, however, were found by them to be accelerated by cyanides, or first accelerated and then depressed. Hahn and Geret (29) reported that HNC retards the activity of a trypsin-like enzyme from yeast. These conflicting results are, I think, reconcilable on the basis of the same suggestion which I have made with reference to the relation of cyanides to chemical reactions,—that whether the reaction shall be accelerated or retarded depends upon the nature of the compound formed with cyanides and hence upon the nature of the reacting material, in this case, the enzymes. The accelerating effect of dilute solutions of cyanide and the retarding effect of stronger solutions on some enzymes is suggestive since it resembles the effect of cyanides, narcotics and many toxic substances on intact organisms, and has also been observed in purely chemical reactions.

Numerous instances are at hand of the depressing and retarding effects of the cyanides upon physiological processes in general; in most cases, these effects can be most reasonably referred to depression of oxidation. The symptoms of cyanide poisoning in vertebrates markedly resemble those of asphyxia. Geppert (6) has described in detail the symptoms in mammals; the respiration is first greatly increased in rate and depth; defecation, vomiting and loss of muscular control follow; breathing becomes more difficult but is still rapid; violent convulsions occur; finally the convulsions cease, the respiration slows down and death occurs with a final increase in respiratory rate. Dontas (30) reports similar symptoms in frogs poisoned with cyanide: irregularity of respiration followed by a marked decrease in rate; tremblings of the muscles; cessation of breathing and disappearance of reflexes. According to Drzewina (31) fish exhibit all of the symptoms of asphyxia when placed in cyanide solutions; they swim violently, come to the surface for air and finally die with decreased respiration. In vertebrates then the symptoms chiefly concern the central nervous system, a fact of particular significance in view of the high metabolic rate of the nervous system which renders it peculiarly susceptible to asyphyxiating substances. Particular emphasis has been placed upon the depressing effect of cyanide upon the respiratory center but this is misleading, as all portions of the brain are affected, as evidenced by vomiting (vomiting center), loss of muscular control (cerebellum?), loss of consciousness (cerebral hemispheres), disappearance of the corneal reflex (sensory nucleus V, motor nucleus VII), etc. Grove and Loevenhart (32) and Gasser and Loevenhart (33) have studied the effect of cyanide upon the medullary centers of mammals. They found that small doses of cyanide stimulate these centers, larger doses first stimulate and then depress. The respiratory center is most sensitive, vasoconstrictor center next and cardio-inhibitory center least sensitive to cyanide. Adding carbon monoxide, hydrogen or nitrogen to the inspired air, clamping the carotid arteries, stopping the artificial respiration or any method which reduces the oxygen supply to the brain produces effects identical with those due to injecting cyanide; that is, cyanide acts in the same manner as lack of oxygen. Loeb in many of his studies has proved that the effect of cyanide on sea-urchin eggs is identical with lack of oxygen: for example, cytolysis of sea-urchin eggs after exposure to hypertonic solutions is prevented by potassium cyanide or an atmosphere of hydrogen (34); fertilized eggs will not develop either in an atmosphere of hydrogen or in the presence of KNC (35), a 1/2000 per cent solution completely but reversibly preventing development, while weaker solutions only retard it; cyanide prevents the parthenogenetic action of hypertonic sea water, for which oxygen is likewise necessary (35); unfertilized eggs are much less susceptible to cyanide than fertilized and developing ones (35), and as is well known consume much less oxygen than the latter; the toxic effects of a number of solutions upon sea-urchin eggs can be prevented either by withdrawing oxygen from the toxic solutions or adding cyanide to them (36), (37). The circulation of the protoplasm in plant cells, for which oxygen is necessary, is reversibly stopped by hydrocyanic acid (38). The body temperature of mammals, which is due to oxidation, falls reversibly in non-lethal cyanide poisoning (39). Amberg, Loevenhart and McClure (40) have observed that certain experimentally induced inflammations are greatly benefited or prevented by the application of substances which yield oxygen readily, and cyanide aggravates the inflammation and counteracts the curative effects of these substances. Dontas (30) has made an interesting study of the action of sodium cyanide upon the nerves and muscles of the frog. He found the central nervous system to be most susceptible, the peripheral nerves less susceptible and the muscles the least susceptible of the neuromuscular apparatus. The conductivity of nerves was found by him to be completely but reversibly abolished in the proper concentrations of cyanide. The current of action and the current of injury completely disappear in nerves immersed in cyanide of proper strength, and return to their former value when the cyanide is washed out. I have discussed elsewhere the relation of these currents to metabolic rate (41). Strong sunlight is injurious or even fatal to many of the lower invertebrates, presumably because it accelerates metabolic processes too greatly; Drzewina (31) has found that these effects of light are abolished in the presence of cyanide. Moore and Willaman (19) state that photosynthesis is decreased in plants fumigated with HNC gas and, as is well known to plant physiologists, is similarly affected by lack of oxygen.[2] They further found that cyanide interferes with the consumption of sugar in plants, presumably an oxidation. According to Skanischewsky (42), cyanides decrease the power of animals to perform simple oxidation such as the formation of phenol from benzene. Budgett (43) has noted that lack of oxygen and potassium cyanide (also other poisons) produce identical structural and death changes in Protozoa; and he figures a disintegration gradient in *Oxytricha* in lack of oxygen and cyanide which is identical with the gradients which we have described for many of the lower organisms killed in cyanide. Planaria also exhibits the same death gradient in lack of oxygen as it does in cyanide. Cyanide prevents the germination of seeds and fungus spores, and completely inhibits their growth (13), (17), (44); dilute solutions may accelerate these processes. For growth and germination of such materials, oxygen is of course indispensable.

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[2] Statements of this kind are made on the authority of Jost's *Plant Physiology*.

These instances, including a variety of physiological processes, all strongly suggest that there is a direct relation between cyanide and metabolic rate. The action of cyanide upon these processes is similar and often identical with the effect of absence of oxygen; and is most striking upon processes and conditions known to require much oxygen.

It must always be borne in mind that the concentration of cyanide employed in such experiments is of the greatest importance, since dilute solutions often produce the opposite effect from stronger solutions.

The final group of data which I wish to present includes those instances in which direct measurements of the respiratory exchange in the presence of cyanide have been made. This kind of evidence is of course the most convincing and I have therefore made an effort to collect all of the cases of this kind extant in the literature. The impetus to experimentation upon this subject was given by the discovery of Claude Bernard (45) that the venous blood is red in cyanide poisoning. This was confirmed by Gaethgens (46) who notes that in cyanide poisoning in rabbits the blood in the small vessels of the ears and mucous membrane of the lips and in the jugular vein is bright red, and that the venous side of the heart (as viewed through an opening made in the diaphragm) is of the same bright red color as the arterial half. The observation has of course been made repeatedly since.[3] Claude Bernard did not explain the red color; but with his usual perspicuity he determined that the action of cyanide upon the blood is not the same as that of carbon monoxide, since blood when mixed with cyanide will not turn red unless air is present. In other words, in all probability, the red color is simply due to oxyhemoglobin. Hoppe-Seyler, Preyer and their students were much interested in this question of the causation of the red color of the venous blood in cyanide poisoning, and they believed it was due in part at least to the formation of a compound between cyanogen and hemoglobin. But Hoppe-Seyler (48) was unable to prove that any such compound exists in the circulating blood when cyanide is present, since such blood gives the same spectrum as normal blood and yields normal hemoglobin crystals. This question was settled by the experiments of Zeynek (49) who proved that hemoglobin will not unite with cyanide, and oxyhemoglobin unites with it only after heating several hours at body temperature; and it is now universally recognized that the action of cyanide on vertebrates is not due to such union.

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[3] Chio (47) presents a theory that the red color of the venous blood in cyanide poisoning is due to the action of cyanide on the walls of the veins so that the venous blood flows so fast through the veins that the cells do not have time to withdraw oxygen from it! The idea seems absurd, as there is no evidence of vasomotor fibers in the walls of the smaller veins. Chio's further contention that cyanide acts by disturbing the neutrality mechanism is pertinent only to the higher animals, whereas cyanide produces similar effects on all animals.

The earlier experiments on the rate of oxygen consumption in cyanide poisoning were inconclusive because of inadequate technique. The first reliable experiments were those of Geppert (6), who pointed out the inadequacies of the earlier methods and used improved methods. Geppert found that the oxygen consumption of mammals is increased during the first stages of cyanide poisoning and later falls markedly. When all of the symptoms of poisoning have developed, the oxygen consumption is always less than normal, in spite of the fact that the animal is in violent muscular convulsions and that the respirations are greatly increased in rate and depth. It is quite impossible therefore to ascribe the decrease in oxygen consumption to effects upon the respiratory center, for the decrease appears while the respirations are enormously accelerated and the amount of air passing through the lungs is much greater than under normal conditions. It was further conclusively proved by Geppert that the red color of the venous blood is due to oxyhemoglobin. Such red venous blood was found by him on analysis to contain as much or nearly as much oxygen as the arterial blood, and the oxygen content of the arterial blood was the same as usual. The arterial blood therefore takes up oxygen in the lungs in the usual amount but the tissues do not withdraw this oxygen. Geppert further demonstrated that the oxygen in the venous blood in cyanide poisoning is not held or bound in any other than the normal way, since the oxygen can easily be withdrawn from the blood, as by allowing the animal to breathe hydrogen. After recovery of the animals from non-lethal doses of cyanide, the oxygen consumption and the color of the venous blood return to normal. I believe that anyone who will take the trouble to read Geppert's paper carefully will be convinced that he has avoided every source of error and answered all objections, and that there is no escape from his conclusion that in the animals with which he worked the oxygen consumption of the cells is directly decreased in the presence of cyanide.

A considerable number of similar experiments have been performed since on various living materials which entirely support Geppert's conclusion. Schroeder (50) measured the effect of KNC upon the rate of oxygen consumption and carbon dioxide output in *Aspergillus.* He found that the oxygen consumption is reduced 50 to 94 per cent according to the concentrations used, and the carbon dioxide production also greatly diminished. Recovery and return to the original rate of respiratory exchange occurred from all except the strongest concentrations. Skanischewesky (42) determined the action of a number of nitrils on mammals and found that all of them decreased both the rate of oxygen consumption and of carbon dioxide production. Complete recovery occurred. Since the effect of the various nitrils was the same, the author concluded that the NC group was responsible for the result. Vernon (51), working on isolated kidneys perfused with Ringer's solution, found that the oxygen consumption of such preparations is greatly decreased when HNC is added to the Ringer's solution and gradually rises again when the cyanide is washed out. Batelli and Stern (52) studied the respiratory exchange of minced organs and tissues of vertebrates, and found that such preparations consume oxygen and give off carbon dioxide in large quantities for several hours after death, the respiratory exchange of course gradually diminishing. They determined the action of potassium cyanide on beef liver and muscle, horse muscle and brain and pigeon muscle, performing sixteen experiments in each of which one to five concentrations of cyanide ranging from 1/12 to 1/10,000 mol. were employed. In all cyanide-containing solutions, a decrease in both oxygen consumption and carbon dioxide production was observed, the amount of decrease depending upon concentration. Whether recovery occurs was not stated by the authors. Warburg (53) found that the oxygen consumption of sea-urchin eggs and red blood corpuscles of geese is decreased 50 to 70 per cent in the presence of potassium cyanide. War burg (54), Fiechter (12) and others noted that the oxygen consumption of yeast is decreased by cyanide and nitrils. Loeb and Wasteneys (55) also demonstrated that cyanide decreases the rate of oxygen consumption in sea-urchin eggs. In sponges,[4] I showed that dilute solutions of KNC increase the rate of oxygen consumption, slightly stronger ones first increase it and then decrease it, and stronger solutions decrease the rate to a marked degree (1). In all of my experiments complete recovery and return to original rate of oxygen consumption was observed. Onaka (56) measured the oxygen consumption of red blood corpuscles of geese in cyanide. In 1/1000 mol. solution no oxygen was consumed; in 1/10,000 mol., the oxygen consumption was about 20 per cent of the normal; and in 1/20,000, about 40 or 50 per cent of the normal. The result was reversible, recovery occurring in about one and a half hours. Onaka further noted that cyanide does not affect the oxygen-carrying function of the red blood corpuscles, since corpuscles in cyanide will lose their red color when hydrogen is bubbled through, and regain it when oxygen is again admitted. Shafer (57) found that hydrocyanic acid gas (and other volatile insecticides) decreases the rate of oxygen consumption and carbon dioxide output in *Passalus cornutus*, a common Lucanid beetle.

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[4] I take this opportunity of correcting an erroneous statement made in that paper regarding the taxonomic position of Suberites, the sponge employed. It is not a calcareous sponge, as stated, but a siliceous sponge, belonging to the Monaxonida.

These experiments, covering a wide range of materials and performed independently by a number of investigators, furnish a sound basis for the conclusion which has been generally drawn from them that cyanides directly decrease the rate of oxygen consumption of protoplasm. The chief objection raised against this conclusion is the one advanced by Carlson (58) and others,—that anaerobic organisms and tissues are just as susceptible to cyanide as aerobic ones. To this objection there is, I think, an adequate answer. The energy-producing processes of anaerobic organisms are not in reality different from those of aerobic ones. Whereas the latter employ extramolecular oxidations, the former make use of intramolecular ones, such as the reduction of sulphates, nitrates, etc. A case in which the evidence is complete is that of yeast. Yeast can consume oxygen in the ordinary way, deriving energy from ordinary protoplasmic respiration; it can also carry out an intramolecular oxidation, the splitting of sugars into alcohol and carbon dioxide. The latter process is in fact accelerated by lack of oxygen, showing that it can take the place of ordinary respiration. Cyanides by virtue of their reducing powers ought to interfere with both types of oxidations; this is indeed the case, as was long ago pointed out by Fiechter (12). In fact the intramolecular oxidation is much more sensitive to cyanide than the extramolecular one, perhaps because the former is a simpler reaction. It is worthy of note in this connection that cyanides also interfere with the oxidations of sugar in vitro, as by Fehling's and similar solutions.

Since cyanides decrease the rate of oxygen consumption, it follows that cells or organisms or parts of organisms which have the highest rate of respiratory exchange will be more susceptible to cyanide and will therefore die in lethal concentrations of cyanide faster than parts or organisms respiring less actively. On this basis the death gradients in organisms and the differences in time of death of different individuals or parts of organisms killed in cyanide have been interpreted by workers in this laboratory (3) as due to differences in metabolic rate, or more specifically, rate of oxygen consumption. This interpretation, therefore, was neither invented, devised nor assumed by these workers, as certain critics of it persist in saying, but was simply the logical conclusion to be drawn from the large mass of experimental evidence which I have summarized here. In fact, potassium cyanide was chosen by Child (59) for the purpose of demonstrating such gradients after he had become convinced from other evidence that they existed because cyanide was generally accepted by physiologists and pharmacologists as a depressor of metabolic processes. We have however tried wherever the nature of the material permitted to check the susceptibility data by direct measurements of carbon dioxide production (determinations of oxygen consumption will be made next). The carbon dioxide production has been determined by the biometer and by Haas's indicator method (60). In this way Child has proved that individuals or parts of Planaria which are more susceptible to cyanide also invariably give off more carbon dioxide than less susceptible ones.[5] All conditions which are known to increase oyxgen consumption and carbon dioxide output in organisms, such as motor activity, injury, growth and regeneration, rise of temperature, also increase the susceptibility to cyanide. Young and growing regions of plants and animals, or young organisms carry on metabolism more actively than older ones and are likewise more susceptible to cyanide. The same facts are known for developing eggs, the oxygen consumption and heat production and also susceptibility to cyanide increasing from fertilization up through the gastrula stage (61). Alvarez (62) has found the same conditions to obtain in the digestive tract. The upper portions of the intestine have a greater inherent irritability, a faster rate of contractile rhythm, contain more catalase, give off more carbon dioxide and are more susceptible to cyanide than lower regions. Allee (63) has determined similar facts with regard to isopods. In these organisms, a number of conditions which are known to accelerate metabolic rate were shown to increase the positive rheotactic response, the susceptibility to cyanide, and the carbon dioxide production (64).

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[5] A special statement is required with regard to the conditions in starvation and feeding. Starvation gradually increases the susceptibility to cyanide. However, the total metabolism, as determined by CO_2 production, decreases during the early stages of starvation and increases only in the later stages. This apparent discrepancy between susceptibility and metabolic rate is quite simply explained. Susceptibility is commonly measured by the time of death of the surface of the body, little attention being paid to the digestive tract. Total metabolism, however, involves also the metabolism of the digestive tract which, as is well known, is greatly increased by feeding; this increase is quite noticeable in Planaria also. Therefore, in the first stages of starvation, as the activity of the digestive tract is diminished, the total metabolism falls, whereas the susceptibility to cyanide (of the body surface) remains the same or increases. In the later stages of starvation, both susceptibility and total metabolism increase. The increased activity of the digestive tract through feeding can be detected by cyanide also, provided one's attention is directed to this particular point, that is, the digestive tract often disintegrates in fed animals before the body wall does. The term susceptibility, however, as used by us, generally refers to the susceptibility of the surface only. The full report of Child's work on starvation and feeding is now in press in this journal.

The idea has been advanced by some workers, recently again by B. L. Lund (65), that differential susceptibility to cyanide is due to differential permeability. There has never been the slightest doubt in the minds of either Professor Child or myself that a relation exists between permeability and susceptibility to cyanide; we have certain ideas as to the nature of this relation but have not yet subjected them to experimental proof. The explanation given by B. L. Lund, however, that differential susceptibility is due merely to an easier penetration of cyanide into more permeable regions, is altogether too simple and quite untenable in the face of the following facts. *a*, The same susceptibility gradients appear in organisms killed with neutral red or other colored substances as in cyanide. In these cases the colored substance can be observed entering the cells. All cells or parts in many cases stain equally red, proving that the dye has penetrated throughout; nevertheless death occurs as a gradient identical with the gradient in cyanide. *b*, Death gradients like those in cyanide occur in organisms killed by lack of oxygen or extreme temperatures. *c*, The death gradient in dilute solutions of cyanide or other reagents is exactly the reverse of that obtained in strong concentrations. *d*, The intestine of a recently fed Planaria is often more susceptible to cyanide than the body wall; in other words, the cyanide *passes through the body wall, leaving it intact*, and attacks the intestine. *e*, Acids are generally believed to decrease permeability; nevertheless, non-lethal concentrations of acids, unless very dilute, increase the susceptibility to cyanide.

In view of the fact that Lund's experiments are the only ones on record in which quantitative measurements have failed to show a decrease in the rate of oxygen consumption in the presence of cyanide, it is necessary to consider his material and methods. Both are objectionable in several respects. In the first place, Paramecium is very resistant to cyanide; in fact, it is the most resistant animal, except Amoeba, which we have encountered. It is therefore not to be expected that cyanide in concentrations ordinarily employed would greatly affect the rate of oxygen consumption. It is therefore necessary to use high concentrations of cyanide to produce any effect, and this Lund has not done, since by his method the animals must be exposed to the cyanide for long periods of time, with the result that relatively dilute solutions have to be employed. In his table 3, where the strongest solutions are employed, he happened to use a particularly resistant lot of Paramecia. It seems quite probable that most of the concentrations used by Lund were too dilute to produce a measurable effect. This is further indicated by the fact that in three of the four columns of determinations given, the oxygen consumption of Paramecium was accelerated in the strongest solutions used. Such acceleration occurs only in solutions relatively dilute for the organism concerned, and it indicates that these strongest solutions are to be regarded as dilute for Paramecium, and the others as too dilute to produce any effect. It is further possible that the alkalinity of the cyanide may have a stimulating effect. The oxygen consumption of Protozoa is so slight that by Lund's method it is necessary to inclose the organisms for many hours in small bottles. While this procedure might be satisfactory under normal conditions, it is certainly objectionable where a drug is present because the initial effect of the drug might be quite the opposite of the final effect, and the oxygen determination at the end of a number of hours (10 to 51 in Lund's experiments) is really meaningless, since it is the average of all of the changes which have ensued in the bottle during the experiment. Lund analyzes the bottles with the Paramecia still inclosed in them, so that the error due to the iodine absorption by the dead animals is relatively large. Thus in his table 2, the error due to iodine absorption is actually one-fourth to one-third of the apparent oxygen consumption. Furthermore, the iodine absorption is measured only at the beginning of the experiment; Lund does not know what it is at the end of the experiment, that is, the control and the experimental bottles are not really comparable. Lund says that Paramecia do not alter the iodine absorption of water but as he gives no reasons in support of this statement, it must be regarded as pure assumption. I do not think it is really possible to determine the iodine absorption in the presence of cyanide in bottles of different oxygen content, and hence this source of error which is considerable in Lund's experiments cannot be controlled by his method. One would further like to know what effect upon the oxygen consumption, the production of CO_2 and other metabolic products may have in organisms respiring for hours in small closed bottles; and how the increasing acidity of the water may alter the equilibrium conditions with cyanide. There is no doubt that decreasing the alkalinity also decreases the susceptibility to cyanide. It is also possible that acclimation to cyanide is involved in the longer periods of exposure. One notices that the animals respire relatively less in the longer periods than in the shorter ones; although this fact is neither mentioned nor explained by Lund, it is presumably due to starvation, although it might also be due to depression from the accumulation of metabolic products in the water.[6] In fact there are so many sources of difficulty involved in the method used that the results cannot be interpreted with certainty. A final great lack in Lund's experiments is that no figures are given anywhere of the normal rate of oxygen consumption of the same lots of Paramecia. In reference to one table, Lund says that the average oxygen consumption was found to be the same in tap water as in cyanide but more definite data upon this point are desirable. It seems to me that it is really essential in such experiments to determine at least once, preferably two or three separate times, the normal rate of oxygen consumption of a particular lot of animals, and then determine the oxygen consumption of the same or a comparable lot of animals in cyanide. It is further desirable that determinations should be made with higher concentrations of cyanide and shorter periods of exposure.

I do not think one can accept Lund's conclusion in regard to table 4, that the cessation of oxygen consumption in cytolysing solutions of KNC is due entirely to the cytolysis and not to the cyanide. We have plenty of evidence that cytolysed and disrupted cells not only use up oxygen but may even use up more oxygen than intact cells (owing to the stimulation of injury). Some of this evidence may be found in Vernon's *Intracellular Enzymes*, pp. 138ff. Thus Batelli and Stern (52) and Harden and MacLean (67) found that a variety of minced organs and tissues of vertebrates use up oxygen and give off carbon dioxide. Warburg's experiments are of great interest. He found (68) that when the plasma membrane of red blood corpuscles is destroyed by freezing and thawing or by distilled water, the cell remnants consume oxygen and give off carbon dioxide at the same rate as intact cells or often more rapidly. Liver extracts containing no intact cells also consume oxygen and give off carbon dioxide (69). Unfertilized sea-urchin eggs rubbed up with sand or broken by shaking or cytolysed by diluted sea water still use up nearly as much oxygen as intact eggs, and by the most favorable methods of preparation may use even more (70). Suspensions of granules obtained from unfertilized eggs respire faster than a corresponding quantity of intact unfertilized eggs. In support of his contention, Lund has quoted only one reference, that to Warburg's work on *fertilized* eggs. If Lund had read Warburg's original paper (70) instead of the general paper to which he refers, he would have found that he has misunderstood Warburg's statements. Warburg's results with fertilized eggs were really these. Fertilized eggs respire several times faster than unfertilized eggs. This additional oxygen consumption due to fertilization is lost when fertilized eggs are broken up by the above methods. Nevertheless such disrupted fertilized eggs respire as much as or a little more than an equal quantity of cytolysed *unfertilized* eggs. In other words, it is only the stimulation due to fertilization which is dependent upon structure and is lost upon cytolysis, the basic metabolism continuing just the same. Lund is therefore left without any support for his contention that oxygen consumption ceases upon cytolysis, except his own experiments with high oxygen pressure. It is, however, manifestly unfair to use oxygen as a cytolysing agent in order to prove that oxygen consumption ceases upon cytolysis. In the absence of further facts with regard to the effect of cytolysis upon oxygen consumption in Paramecium and in consideration of the facts quoted above with regard to other material, one may conclude that the failure of Paramecium to respire after cytolysis by cyanide is due in part at least to the action of the cyanide, and constitutes an indirect proof that cyanide decreases the rate of oxygen consumption in Paramecium. It is manifestly ridiculous for anyone to maintain that fragments of protozoan cells do not respire, since such fragments swim about for a long time and retain contractile power.

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[6] Woodruff (66) has shown that the metabolic products excreted by Paramecium have a depressing effect upon it.

METHOD

The present experiments were performed upon *Planaria dorotocephala*, a turbellarian flatworm common in certain spring-fed pools near Chicago. Large numbers of these animals are kept on hand in the laboratory in covered dish pans. They are fed three times a week on liver. As the city water is chlorinated and has been found injurious to organisms, we use only well water for all stock cultures and experiments. This water is obtained from a well sunk in the basement of the zoölogy building. It is practically oxygen-free, except when the pump sucks a little air in, and oxygen is therefore added to it by running it through a simple aerating device. This brings the oxygen content up to about 3 cc. per liter. For my experiments I have still further aerated it by shaking it in a large bottle, obtaining by this means an oxygen content of 5 cc. or more per liter.

The apparatus used was simple and similar to that employed in my previous experiments along this line. A 1000 cc. Erlenmeyer flask was provided with a tightly fitting rubber stopper perforated with three holes. Through one of these holes was inserted a glass tube reaching to the bottom of the flask through which water was siphoned in; through another a similar tube also reaching to the bottom through which water was siphoned out; and the third hole was used to admit air during the siphoning, being closed at other times by a short glass rod. Screw clamps on rubber tubing inserts were provided at necessary places. The exit tube was bent over and extended several inches below the bottom of the flask. This apparatus can be made air-tight without the slightest difficulty, as proved by the facts that no water would siphon out of it when all other exits were closed as in the experiments, and if it was filled full of warm water, all exits closed, and allowed to cool, the flask would break as soon as the temperature had fallen two or three degrees, owing to the contraction of the water.

For each experiment two or three hundred worms were used. These were selected at random from the general stock and their heads cut off at least several hours before they were to be used. Such decapitated worms remain absolutely quiet, if they are shaded from direct light, throughout the course of an experiment. There is therefore no possibility that differences in motor activity are involved in the results. No difference can be observed in the behavior of these worms in normal and cyanide-containing water.[7] Each lot of worms prepared in this manner was used in several different experiments, being allowed to recover in water between successive experiments. It was thus known that the animals recovered completely and regained their former rate of oxygen consumption after exposure to cyanide.

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[7] Animals which have been exposed to high concentrations, as 1/2000, show on stimulation a decreased ability to respond, and are not as successful in clinging to the substratum as the normal worm. These effects disappear shortly on return to water, and are not observable in concentrations such as 1/25000 which lower the oxygen consumption to a considerable degree. The action of cyanide on the irritability of organisms is a separate problem, which I shall shortly undertake.

The procedure in each experiment was as follows: Several liters of shaken well water were poured into a large elevated receptacle. The worms were put into the Erlenmeyer flask. To prevent them from being carried up the exit tube a piece of well-washed cheesecloth was tied over its end. Water was siphoned from the receptacle into the flask, allowing the latter to overflow for some time so that the worms, the flask and all of the tubes were thoroughly washed. At the same time a sample of the same water was collected from the receptacle in the same manner into a 250 cc. narrow-mouthed bottle with ground glass stopper to serve as control. All openings to the flask were then closed and it and the control bottle were placed in a large pan of water kept constant to 0.5 of a degree, and regulated to the same temperature as the water in the flask. After one hour the flask was taken out and shaken thoroughly to insure an even distribution of its oxygen content. Water was then siphoned from it into a 250 cc. narrow-mouthed bottle, allowing this to run over for some time so as to obtain a sample which had not come in contact with air. This sample and the control sample were than analyzed for oxygen content, the difference between them being, of course, the amount of oxygen consumed by the worms.

This procedure was repeated twice with normal water, so that three separate determinations of the normal rate of oxygen consumption of the same lot of worms were obtained. The desired amount of cyanide was then added to the water in the receptacle (which was of course refilled for each hour determination) and three more separate successive hour measurements were made of the rate of oxygen consumption of the same worms in the presence of cyanide.

The potassium cyanide was weighed out fresh for each hour determination. In the case of the very dilute solutions, where the amount of cyanide to be weighed was so small that absorption of water during the weighing might introduce a considerable error, stronger solutions were made (fresh each time), and the necessary amount of these stronger solutions was poured into the receptacle. As both control and experimental water comes from the same receptacle, they are identical in composition, the control always containing cyanide and the same amount of cyanide when the experimental water contains it. It is quite essential that the control water contain the same amount of cyanide as the experimental water, since cyanide affects the method of oxygen analysis.

It might be supposed that by this method oxygen might enter the water during the taking of the sample, since it is necessary to admit atmospheric air into the flasks during the siphoning. I have found by test, however, that this is not the case and that there is not the slightest error due to this circumstance. I have made triplicate analyses of the same water, two of the three samples being collected by passage through the same Erlenmeyer flasks used in the experiments, and the third sample taken directly into the 250 cc. bottle. The water used in these tests always contained less oxygen, often very much less, than the water encountered in the experiments, including that in which worms had been respiring. The analyses of such triplicate samples showed that they contained the same quantities of oxygen, within 0.01 to 0.04 cc. of oxygen per liter, except in the water with exceedingly low oxygen content where the variation in the three samples ranges to 0.09 cc. per liter. But even this variation cannot be regarded as due to the process of siphoning but rather to the general manipulation, since in each of the two tests conducted with very low oxygen water, one of the siphoned samples is as close to the direct sample as in the cases where water of higher oxygen content was used. It may therefore be safely said that no oxygen is absorbed by water having an oxygen content below that corresponding to a state of equilibrium with the air by siphoning it with admission of air. The data of these tests are given in the accompanying table 1, which includes all of the tests made. The method may therefore be regarded as perfectly adequate for the purpose. In the early part of the work I used an Erlenmeyer flask for control and collected the control sample from it in the same manner as the experimental sample was collected, but as these tests showed that such a procedure is unnecessary, the control sample was thereafter collected directly into the bottle in which the analysis was carried out.

The samples of water collected in this manner were analyzed by Winkler's method, following the procedure given by Birge and Juday (71). The 1/100 normal solutions recommended by them certainly permit of greater accuracy than the 1/40 normal solutions commonly in use. I have also found the suggestions of McClendon (72) helpful. The thiosulphate was kept in a large darkened bottle, provided with a soda-lime tube to keep out carbon dioxide and fed by siphon into a burette provided with a side arm. It was standardized at intervals with permanganate as described in my first paper (1). As a matter of fact, however, the actual normality of the thiosulphate solution is of little consequence provided it does not change appreciably during the titration of all of the samples from one experiment.[8] In most of the experiments, all of the samples were analyzed within a few hours of each other, but in some, owing to the shortness of the days, some of the samples had to be left until the next day to be titrated. As Birge and Juday noted, such delay introduces no error into the titration.

The Winkler method depends upon the setting free of iodine. If anything is present in the water which takes up iodine, an error will consequently be introduced into the results. Do Planarians add any iodine-absorbing material to the water? This can be determined quite simply as follows: Two hundred and fifty cubic centimeters of the water to be tested are acidified with about ½ cc. of concentrated hydrochloric acid, a known amount of N/100 iodine run into it from a burette, and the water then titrated with thiosulphate. The difference between the amount of iodine added and the amount recovered represents the amount of iodine absorbed. In this way I have found that the well water contains some iodine-absorbing material as compared with distilled water blanks, but that well water which has contained worms for one hour has less iodine-absorbing power than the ordinary well

TABLE 1

Tests of the accuracy of the apparatus; analyses in triplicate of the same water, two samples having been passed through the apparatus as in the experiments, and the third sample taken directly

KIND OF WATER	SAMPLE THROUGH APPARATUS	SAMPLE THROUGH APPARATUS	DIRECT SAMPLE
	Oxygen content, cubic centimeters per liter		
Slightly aerated well water	1.28	1.30	1.30
Slightly aerated well water	1.93	1.94	1.90
Standing well water	4.09	4.07	4.09
Standing well water	3.95	3.98	3.97
Standing well water, stirred	4.54	4.55	4.53
Unaërated well water	0.57	0.63	0.54
Unaerated well water	0.51	0.59	0.60

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[8] Thiosulphate solutions to which NaOH has been added as suggested by Birge and Juday show practically no deterioration.

water. That is to say, the oxygen consumption of the worms is actually a little greater than appears from the analysis since in the experimental bottles less iodine is absorbed than in the control bottles. I have not made this correction, since it was somewhat variable, and I thought it not worth while to undertake the great amount of extra labor which would have been required to determine its amount for every sample. The amount of this correction was not large enough to be of any consequence for the general conclusion to be drawn from the experiments.

As I have already pointed out, cyanide has marked iodine-absorbing power. If the above procedure is repeated with water containing cyanide, it will be noticed at once that as the iodine is run into the solution the color fades out to some extent. If such solutions are titrated with thiosulphate, it will be found that it is almost impossible to come to an end point, indicating that a condition of changing equilibrium exists between the various substances present in the solution. Since there is no difficulty in determining the end point by Winkler's method in solutions containing cyanide, it is evident that during the time occupied in performing Winkler's analysis, a condition of static equilibrium is reached. I have not been able to devise any way of finding out what the iodine absorption in cyanide-containing water really is. Since, however, in my experiments, the control water always contains cyanide when the experimental water does, and this is an absolutely essential point, the error due to absorption of iodine by cyanide may be regarded as eliminated. There remains still the possibility that the effect of the worms on the iodine-absorbing power of the water may be different in cyanide than in normal water. Such evidence as I have been able to obtain upon this point indicates that in cyanide also the worms reduce the iodine absorption of the well water. It is therefore likely that all of the figures of the oxygen consumption of the worms are a little too low. The results are, however, so very consistent, definite and striking that it cannot be supposed that these small sources of error could in the least alter the conclusion to which they lead.

EXPERIMENTAL RESULTS

The results of my experiments are recorded in the accompanying tables. These experiments have not been selected for presentation but they represent all that I performed with two or three exceptions where accidental difficulties arose. In every experiment which I carried out, the result was perfectly clear-cut. There were no exceptions. In all the oxygen consumption was decreased, the amount depending upon the concentration of the cyanide. As already stated, each experiment, except in one or two cases, consists of six one-hour measurements of the oxygen consumption, the first three in normal water, the second three in cyanide-containing water, all six being performed on the same worms which remain in the flask throughout the experiment. To save time, two lots of worms have generally been run with one control. The six figures of the oxygen consumption of a lot of worms are given in the vertical columns in the tables. Each figure represents, of course, the difference between the oxygen content of the control bottle and the oxygen content of the sample drawn from the experimental bottle. Several times samples were lost (and if this happened to be the control bottle, two determinations were lost thereby), through accidents of one kind and another; such loss is indicated by a blank in the table. In connection with the most dilute solutions, I measured the oxygen consumption during the first half-hour and second half-hour in cyanide instead of during the first hour, in order to discover whether or not there is any initial stimulating action of the cyanide. In connection with each concentration, some figures of the respiration after recovery are given. Although it was known that recovery always occurred since the same lot of worms was used several times, in fact until the animals had regenerated sufficiently to cause them to move about, the exact figures were not always usable because successive experiments were often performed at different temperatures, and because two lots of worms were always in use simultaneously and I did not always note from which of the two lots a particular set of figures was obtained. Recovery from the higher concentrations required more than 24 hours, with several washings, but occurred in less than 24 hours from the low concentrations. A slight decrease in oxygen consumption in a lot of worms kept several days without feeding will of course occur on account of starvation; for this reason the oxygen consumption after recovery must be expected to be slightly less than it was during the original experiment. This is quite evident when the oxygen consumption is determined more than 48 hours afterwards. In the first two or three experiments the temperature was not kept constant, accounting for a larger variation in successive determinations in those experiments, but with these exceptions, the temperature varied less than 0.5° throughout the course of the experiment.

Extended comment upon the data given in the tables is unnecessary. In all cases, the oxygen consumption is decreased in the presence of cyanide. This decrease ranges from 80 to 90 per cent of the normal in the highest concentrations to 5 to 15 per cent in the lowest. In general the dilute solutions are relatively more effective. Thus, there is little difference in the action of 1/1000, 1/2000 and 1/5000 mol.

TABLE 2

Experiments with 1/1000 mol. KNC

NUMBER OF EXPERIMENT	5a	5b
TEMPERATURE	22°	22°
	O_2 consumed, cubic centimeters per hour	
First hour normal	0.34	0.32
Second hour normal	0.33	0.33
Third hour normal	0.38	0.37
First hour KNC*	0.04	0.08
Per cent decrease	89	77

* It is not possible to expose the worms to 1/1000 mol. solutions for more than one hour, as they disintegrate in two or three hours in solutions of this strength.

TABLE 3

Experiments with 1/2000 mol. KNC

NUMBER OF EXPERIMENT	1	2	13a	13b	18a	18b	25a	25b	27a	27b	33b
TEMPERATURE	Not constant	Not constant	22°	22°	23°	23°	23°	23°	21°	21°	21°
	Oxygen consumed, cubic centimeters per hour										
First hour normal	0.42	0.57	0.39	0.43	0.41	0.45	0.55	0.66	0.79	0.85	0.65
Second hour normal	0.38	0.70	0.47	0.48	0.48	0.49	0.61	0.71	0.87	0.94	0.62
Third hour normal	0.55	0.89	0.44	0.49	0.46	0.44	0.62	0.76	0.82	0.89	0.63
First hour KNC	0.11	0.14	0.07	0.08	0.07	0.08	0.12	0.12	0.14	0.16	
Second hour KNC	0.10	0.13	0.06	0.09	0.05	0.06	0.07	0.08	0.02	0.02	0.06
Third hour KNC	0.06	0.02	0.04	0.07	0.07	0.07	0.08	0.08	0.07	0.09	0.06
Per cent decrease	80	87	87	83	86	85	85	87	91	90	91
			After 48 hours						After 4 days		
Recovery*			0.43	0.45					0.72	0.79	

* Recovery figures are the average of three determinations.

solutions, while the 1/25,000 and 1/50,000 solutions are much more effective than would be expected. With the highest solutions, the cyanide produces its effect so rapidly that the respiration is decreased to its maximum extent by the end of the first hour exposure. In the dilute solutions, however, some little time is required for the depression

TABLE 4

Experiments with 1/5000 mol. KNC

NUMBER OF EXPERIMENT	3a	3b	4a	4b	12a	12b	24a	24b	26a	26b
TEMPERATURE	Not constant	Not constant	19°	19°	23°	23°	21°	21°	21°	21°
	Oxygen consumed, cubic centimeters per hour									
First hour normal	0.49	0.41	0.30	0.35	0.36	0.43	0.73	0.62	0.71	0.68
Second hour normal	0.43	0.34	0.33	0.34	0.38	0.37	0.86	0.76	0.88	0.91
Third hour normal	0.58	0.44	0.29	0.29	0.42	0.45	0.66	0.58	0.83	0.85
First hour KNC	0.17	0.11	0.06	0.06	0.16	0.08	0.19	0.19	0.14	0.24
Second hour KNC	0.21	0.17	0.03	0.03			0.13	0.09	0.13	0.15
Third hour KNC	0.10	0.08	0.09	0.09	0.05	0.06	0.11	0.10		
Per cent decrease	68	68	79	82	70	84	86	81	84	77
					After 48 hours		After 4 days		After 48 hours	
Recovery*					0.43	0.46	0.60	0.71	0.82	0.89

TABLE 5

Experiments with 1/10000 mol. KNC

NUMBER OF EXPERIMENT	6a	6b	7a	7b	11a	11b	29a	29b
TEMPERATURE	23°	23°	20°	20°	23°	23°	21°	21°
	Oxygen consumed, cubic centimeters per hour							
First hour normal	0.57	0.61	0.33	0.37	0.44	0.36	0.76	0.82
Second hour normal	0.43	0.45	0.31	0.30	0.38	0.38	0.76	0.81
Third hour normal	0.53	0.48			0.38	0.43	0.87	0.80
First hour KNC	0.13	0.13	0.09	0.13	0.15	0.14	0.29	0.28
Second hour KNC	0.10	0.11	0.07	0.08	0.11	0.13	0.17	0.44
Third hour KNC	0.13	0.10		0.09	0.09	0.08	0.21	0.11
Per cent decrease	77	78	75	69	71	70	72	66
					24 hours later			
Recovery					0.39	0.41		

to reach its maximum value. This appears in tables 9 and 10, where the oxygen consumption during the first half hour in cyanide shows no depression. In fact, in some of these experiments, 35a, 35b, table 9, and 21a, table 10, there are evidences of an acceleration of the rate of oxygen consumption during the first half-hour in cyanide. However, more experiments with these very dilute solutions would be required

TABLE 6

Experiments with 1/25000 mol. KNC

NUMBER OF EXPERIMENT	8a	8b	10a	10b	14a	14b	28a	28b
TEMPERATURE	23°	23°	22°	22°	23°	23°	21°	21°
	Oxygen consumed, cubic centimeters per hour							
First hour normal	0.40	0.51	0.35	0.32	0.50	0.49	0.67	0.74
Second hour normal	0.36	0.46		0.27	0.46	0.42	0.80	0.79
Third hour normal	0.38	0.46	0.42	0.32	0.44	0.46	0.70	0.79
First hour KNC	0.13	0.25			0.21	0.24	0.41	0.41
Second hour KNC	0.14	0.20	0.22	0.14	0.21	0.24	0.36	0.34
Third hour KNC	0.12	0.21	0.20	0.14	0.21	0.25	0.45	0.40
Per cent decrease	66	55	47	55	55	57	56	51
					After 24 hours		After 24 hours	
Recovery					0.41	0.43	0.79	0.81

TABLE 7

Experiments with 1/50000 mol. KNC

NUMBER OF EXPERIMENT	9a	9b	15a	15b	17a	17b	30a	30b
TEMPERATURE	22°	22°	22°	22°	23°	23°	21°	21°
	Oxygen consumed, cubic centimeters per hour							
First hour normal	0.44	0.33	0.37	0.39	0.42	0.44	0.74	0.66
Second hour normal	0.44	0.33	0.41	0.43	0.44	0.45	0.78	0.81
Third hour normal	0.44	0.32	0.41	0.42	0.43	0.43	0.78	0.79
First hour KNC	0.27	0.22	0.26	0.25	0.31	0.34	0.50	0.54
Second hour KNC	0.24	0.17	0.25	0.26	0.32	0.35	0.46	0.48
Third hour KNC	0.27	0.21	0.24	0.23	0.34	0.34	0.40	0.40
Per cent decrease	42	39	37	41	25	20	41	42
	3 days				24 hours		48 hours	
Recovery	0.39	0.30			0.45	0.46	0.67	0.78

to establish this point. Apparently the action of the cyanide is constant, that is, the respiration soon drops to a certain figure and stays there as long as the same concentration of cyanide is present. There is no cumulative effect of the cyanide. However, one would have to carry on the experiments longer than three hours to establish this point with certainty. It may again be emphasized that the decreased

TABLE 8

Experiments with 1/100000 mol. KNC

NUMBER OF EXPERIMENT	16a	16b	19a	19b	23a	23b	31a	31b	32a	32b
TEMPERATURE	20°	20°	23°	23°	22°	22°	21°	21°	21°	21°
	Oxygen consumed, cubic centimeters per hour									
First hour normal	0.42	0.42	0.37	0.35	0.25	0.27	0.63	0.81	0.55	0.67
Second hour normal	0.48	0.46	0.39	0.40	0.32	0.30	0.67	0.80	0.65	0.67
Third hour normal	0.41	0.37	0.38	0.35	0.27	0.29	0.67	0.74	0.57	0.70
First hour KNC	0.37	0.38	0.37	0.36	0.23	0.24	0.54	0.62	0.50	0.59
Second hour KNC	0.34	0.36	0.29	0.27	0.23	0.20	0.52	0.58	0.40	0.49
Third hour KNC	0.32	0.34	0.33	0.29	0.22	0.21	0.52	0.58	0.43	0.47
Per cent decrease	21	14	13	17	20	24	20	25	27	25
							3 days		48 hours	
Recovery							0.59	0.68	0.56	0.64

TABLE 9

Experiments with 1/150000 mol. KNC

NUMBER OF EXPERIMENT	34a	34b	35a	35b
TEMPERATURE	20.5°	20.5°	20.5°	20.5°
	Oxygen consumed, cubic centimeters per hour			
First hour normal	0.54	0.54	0.54	0.54
Second hour normal	0.57	0.56	0.55	0.54
Third hour normal	0.49	0.49	0.59	0.55
First half-hour KNC	0.27	0.27	0.38	0.38
Second half-hour KNC	0.24	0.24	0.29	0.27
Second hour KNC	0.43	0.42	0.47	0.49
Third hour KNC	0.41	0.42	0.42	0.48
Per cent decrease	16	15	15	8
	24 hours		24 hours	
Recovery	0.56	0.54	0.52	0.53

oxygen consumption in cyanide is in no way related to muscular or ciliary activity, since the animals are perfectly quiet and motionless throughout the course of the experiments.

In a paper which accompanies this one, Child gives the results of his determinations of the carbon dioxide output of Planarians which have been exposed to cyanide. The carbon dioxide production was decreased after exposure to cyanide. That cyanide decreases the respiratory exchange in Planaria must therefore be regarded as an established fact, and conclusions which have been drawn by workers in this laboratory through the use of cyanide as a depressing agent thereby receive support of the most convincing kind.

TABLE 10

Experiments with 1/200000 mol. KNC

NUMBER OF EXPERIMENT	21a	21b	36a	36b
TEMPERATURE	22°	22°	20.5°	20.5°
	Oxygen consumed, cubic centimeters per hour			
First hour normal	0.30	0.35	0.48	0.50
Second hour normal	0.34	0.34	0.54	0.56
Third hour normal	0.29	0.32	0.56	0.51
First half-hour KNC	0.18	0.17	0.26	0.26
Second half-hour KNC	0.13	0.13	0.26	0.29
Second hour KNC	0.29	0.28	0.40	0.42
Third hour KNC	0.29	0.30	0.43	0.44
Per cent decrease	5	13	15	11
	24 hours			
Recovery	0.32	0.32		

SUMMARY

1. An extensive review of the literature on the chemical, physiological and pharmacological action of the cyanides is given. All of this literature supports the generally accepted opinion that the cyanides depress physiological processes in general, and rate of oxygen consumption in particular.

2. A large number of experiments are presented which prove that the oxygen consumption of Planaria is decreased in the presence of potassium cyanide. The amount of decrease depends upon the concentration of the cyanide, ranging from 80 to 90 per cent in 1/2000 mol.

KNC to 5 to 15 per cent in 1/200,000 mol. solutions. The decrease is independent of muscular or ciliary activity. It is entirely reversible, the animals being wholly uninjured by the cyanide and returning to their normal rate of oxygen consumption when the cyanide is washed out of them.

3. Conclusions drawn by workers in this laboratory from experiments on Planaria in which cyanide was used as a depressing agent and in which the results were interpreted on the basis that cyanide is a general protoplasmic depressant, therefore receive convincing support.

BIBLIOGRAPHY

(1) Hyman: This Journal, 1916, xl, 238.
(2) Lund: This Journal, 1918, xlv, 365.
(3) Child: Senescence and rejuvenescence; Individuality in organisms, 1915, Chicago.
(4) Nef: Liebig's Annal., 1892, cclxx, 267.
(5) Bredt and Kollen: Liebig's Annal., 1897, ccxciii, 338.
(6) Geppert: Zeitschr. f. klin, Med., 1889, xv, 208.
(7) Mathews and Walker: Journ. Biol. Chem., 1909, vi, 29.
(8) Curtman and Kaufman: Journ. Amer. Chem. Soc., 1918, xl, 914.
(9) Loevenhart: Berl. deutsch. chem. Gesell., 1906, xxxix, 130.
(10) Kastle and Loevenhart: Amer. Chem. Journ., 1903, xxix, 397, 563.
(11) Kastle and Loevenhart: Amer. Chem. Journ., 1901, xxvi, 539.
(12) Fiechter: Über den Einfluss der Blausäure auf Fermentvorgänge, 1875, dissert. Basel. Also in Maly's Jahrb. f. thier. Chem., v, 269.
(13) Schönbein: Zeitschr. Biol., 1867, iii, 140.
(14) Loew: U. S. Dept. Agric., Bull. no. 3, 1900.
(15) Burge: Sci., 1918, xlviii, no. 1239, 327 (gives references in detail).
(16) Appleman: Md. Agric. Exper. Sta., 1915, Bull. 191.
(17) Schär: Festsch. f. Nägeli und Kölliker, 1891, Zürich, 123.
(18) Jacobson: Zeitschr. f. physiol. Chem., 1892, xvi, 341.
(19) Moore and Willaman: Journ. Agric. Research., 1917, xi, 319.
(20) Shafer: Mich. Agric. Exper. Sta., 1915, Tech. Bull. xxi, 63.
(21) Raubitschek: Wien. klin. Wochenschr., 1912, xxv, 149.
(22) Schultze: Münch. med. Wochenschr., 1909, lvi, 167.
(23) Butkewitch: Zeitschr. f. physiol. Chem., 1901, xxxii, 1.
(24) Vines: Ann. of Bot., 1897, xi, 563.
(25) Mendel and Blood: Journ. Biol. Chem., 1910, xviii, 177.
(26) Frankel: Journ. Biol. Chem., 1917, xxxi, 201.
(27) Abderhalden, Cammerer and Pincussohn: Zeitschr. f. physiol. Chem., lix, 293.
(28) Chittenden and Allen: Studies from Lab. Physiol. Chem., Sheffield Scien. School, 1884–5, 87.
(29) Hahn and Geret: Zeitschr. f. Biol., 1900, xl, 117.
(30) Dontas: Arch. exper. path. u. pharm., 1908, lix, 430.

(31) DRZEWINA: C. R. Soc. Biol., 1911, lxx, 758.
(32) GROVE AND LOEVENHART: Journ. Pharm. Exper. Therap., 1911, iii, 13.
(33) GASSER AND LOEVENHART: Journ. Pharm. Exper. Therap., 1913, v, 239.
(34) LOEB: Pflüger's Arch., 1906, cxiii, 487.
(35) LOEB: Biochem. Zeitschr., 1906, i, 183.
(36) LOEB: Biochem. Zeitschr., 1910, xxix, 80.
(37) LOEB: Sci., 1910, xxxii, 411.
(38) MAYER: Landwirt. Versuchsstat., 1879, xxiii, 335.
(39) HOPPE-SEYLER: Virchow's Arch., 1867, xxxviii, 435.
(40) AMBERG, LOEVENHART AND MCCLURE: Journ. Pharm. Exper. Therap., 1917, x, 209.
(41) HYMAN: Sci., 1918, xlviii, 518.
(42) SKANISCHEWESKY: Dissertation, St. Petersburg, 1913; see also Centralbl. f. Biochem. u. Biophysik, 1913, xv, 780.
(43) BUDGETT: This Journal, 1898, i, 210.
(44) TOWNSEND: Md. Agric. Exper. Sta., 1901, Bull. no. 75, 183.
(45) BERNARD: Lecons sur les effets des Substances Toxiques, etc., 1857.
(46) GAETHGENS: Hoppe-Seyler's Medicin. chem. Untersuch, 1866, iii, 325.
(47) CHIO: Arch. Ital. Biol., 1914, lxi, 1.
(48) HOPPE-SEYLER: Virchow's Arch., 1867, xxxviii, 435.
(49) ZÆYNEK: Zeitschr. f. physiol. Chem., 1901, xxxiii, 426.
(50) SCHROEDER: Jahrb. f. wiss. Bot., 1907, lxiv, 409.
(51) VERNON: Journ. Physiol., 1906, xxxv, 53.
(52) BATELLI AND STERN: Journ. d. physiol., 1907, ix, 228, 410.
(53) WARBURG: Zeitschr. f. physiol. Chem., 1910, lxx, 413; also lxvi, 304.
(54) WARBURG: Münch. med. Wochenschr., 1912, lix, 2550.
(55) LOEB AND WASTENEYS: Biochem. Zeitschr., 1911, xxxvi, 345.
(56) ONAKA: Zeitschr. f. physiol. Chem., 1911, lxx, 433.
(57) SHAFER: Mich. Agric. Exper. Sta., 1911, Tech. Bull. no. 11, 1.
(58) CARLSON: This Journal, 1907, xix, 223.
(59) CHILD: Journ. Exper. Zoöl., 1913, xiv, 153.
(60) HAAS: Sci., 1916, lxiv, 105.
(61) CHILD: Sensecence and rejuvenescence, 1915, Chicago.
(62) ALVAREZ AND STARKWEATHER: This Journal, 1918, xlvi, 186.
(63) ALLEE: Journ. Exper. Zoöl., 1914, xvi, 397.
(64) ALLEE AND TASHIRO: Journ. Animal Behav., 1914, iv, 202.
(65) LUND: Biol. Bull., 1918, xxxv, 211.
(66) WOODRUFF: Journ. Exper. Zoöl., 1911, x, 556.
(67) HARDIN AND MCLEAN: Journ. Physiol., 1911, xliii, 34.
(68) WARBURG: Zeitschr. f. physiol. Chem., 1911, lxx, 413.
(69) WARBURG: Pflüger's Arch., 1913, cliv, 599.
(70) WARBURG: Pflüger's Arch., 1914, clviii, 189.
(71) BIRGE AND JUDAY: Wis. Geol. and Nat. Hist. Survey Bull. 22, 1911.
(72) MCCLENDON: Journ. Biol. Chem., 1917, xxxii, 275.

THE EFFECT OF CYANIDES ON CARBON DIOXIDE PRODUCTION AND ON SUSCEPTIBILITY TO LACK OF OXYGEN IN PLANARIA DOROTOCEPHALA

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SUSCEPTIBILITY IN RELATION TO PHYSIOLOGICAL CONDITION

The following study of carbon dioxide production and susceptibility to lack of oxygen in the flatworm, *Planaria dorotocephala*, as influenced by cyanides is presented as a contribution to the problem of the relation between susceptibility and physiological or metabolic condition. Extensive studies on susceptibility to cyanides and to many other agents, including various anesthetics, acids, alkalies, salts, several alkaloids, "vital" dyes, H_2O_2 and certain physical conditions such as lack of oxygen and high and low temperatures, have been made on a great variety of organisms, including algae (11), (13), (14), protozoa (3), (19), *Planaria* (2), (4), (5), (7) and various other flatworms, developmental stages of starfish and sea-urchin (6), (9), (12) and annelids (16), (17) and data not yet published on various species of coelenterates, flatworms, echinoderms, arthropods and vertebrates. These studies on susceptibility have consisted in determining the survival time or the degree of inhibition of developmental or other processes in higher concentrations and the ability to become acclimated or acquire tolerance in lower concentrations, in relation to region of body (2), (3), (6), (8), (9), (11), (12), (13), (14), (16), (17), (19), physiological age and nutritive condition (4), (7), stimulation by various means (5), and the direction of functional conduction of stimuli (15). The existence of definite graded differences in susceptibility in relation to the physiological body-axes has been demonstrated for all axiate organisms examined, and it has been found that these susceptibility gradients correspond to characteristic differences in rate of development along an axis, and a corresponding gradient in protoplasmic structure is also very generally present in early developmental stages (8). These axial susceptibility gradients also afford a basis for extensive control and modification of form and proportion in ontogeny (12), (16) and regulation (10), since the graded difference in susceptibility along an axis makes possible a graded inhibition, acceleration, acclimation or recovery along that axis. The experimental data already obtained constitute a rational physiological basis for the interpretation of many forms of teratological development.

In an earlier paper Dr. L. H. Hyman (17) suggested that a relation between the differences in electric potential which give rise to the bio-electric currents and the differences in susceptibility exists and recently Doctor Hyman and Mr. A. W. Bellamy have been able to show in this laboratory not only that this relation between regional differences in susceptibility and in electric potential actually does exist, but also that there is a relation between the chief susceptibility gradients and the galvanotactic reaction of animals, orientation occurring with the region or regions of highest susceptibility toward the cathode. The region of highest susceptibility is electro-negative through the galvanometer to regions of lower susceptibility, i.e., the region of highest susceptibility is electro-positive internally to other regions and in galvanotactic orientation this region is directed toward the cathode. Except for a brief article by Doctor Hyman in *Science* (27), these data on the relation between susceptibility gradients, galvanotaxis and bio-electric currents are as yet unpublished.

Certain minor differences appear in the susceptibility relations between different body-regions or organs, as determined by different agents or conditions, particularly in the later stages of development of the higher animals, where differentiation of different organs is relatively great. The important fact, however, is the very high degree of uniformity in susceptibility relations, particularly in the earlier stages of development and in the lower organisms, both plant and animal, to certain ranges of concentration or intensity of a great variety of agents. This fact indicates that the susceptibility of protoplasm to the action of external agents within certain limits of concentration or intensity is primarily an expression of some very fundamental factor in physiological condition.

It has been suggested by some that susceptibility depends merely on permeability of membrane and that the apparent differences in susceptibility are simply differences in permeability to the agents employed. While there can be no doubt that permeability is a factor in determining the relation between protoplasm and its environment, permeability alone will not serve to account for the facts of susceptibility. In the first place, the regions of the body which are most susceptible to high concentrations or intensities are likewise able to acclimate or acquire tolerance most rapidly and most completely to a certain range of low concentrations or intensities (1), (2), (7, chap. iii), (12), i.e, not merely the penetration of the agent but the physiological activity of the living protoplasm is concerned in susceptibility. Second, the susceptibility relations, e.g., along an axis, to certain extreme temperatures and to lack of oxygen are the same as the susceptibility relations to chemical agents in certain concentrations. In the case of temperature and lack of oxygen the effect is obviously not a matter of penetration of a chemical substance through a membrane. And finally, it must be remembered that permeability of the plasma membrane itself is not a fixed condition established independently of other conditions in protoplasm, but changes with changes in protoplasmic conditions. It is becoming more and more evident that the plasma membrane consists of living protoplasm, and much recent work on permeability emphasizes the importance of chemical reaction in relation to permeability. (See for example (20, p. 443); (21, p. 368); (22, p. 366); (23)).

The high degree of uniformity and the absence of specificity in the general susceptibility relations to a great variety of agents, including cyanides, alcohol, ether, various urethanes, salts, acids, alkalies, alkaloids, vital dyes, temperature and lack of oxygen within certain limits of toxic concentration appears in various ways. For example the general susceptibility gradients along the body axes of the individual, as indicated either by the progress of death in lethal concentrations, by the degree and rapidity of acclimation or recovery in or after lower concentrations, or by the differential effect on the rate and course of embryonic development and regulation and on various functional activities, are essentially the same for all these agents and conditions. Similarly, differences in susceptibility in different individuals, corresponding to differences in physiological age, nutritive condition, etc., also show the same uniformity with the different agents and conditions. It is perhaps necessary to emphasize the fact that this uniformity in susceptibility relations does not in any sense imply that the action on living protoplasm of all these agents is of exactly the same sort at all concentrations or intensities. It is merely a statement of the fact that within certain limits of toxic concentration or intensity the regional and individual differences in susceptibility are similar. Many agents, e.g., the anesthetics, produce in low concentration or with short period of action temporary, readily reversible effects, while with higher concentration or longer time the effects are less readily reversible or irreversible and finally end in death. So far as differences of this sort exist, it is the less readily reversible or irreversible changes which are primarily concerned in these general susceptibility relations, and the relations may or may not be the same for the readily reversible changes.

The action of benzamid will serve as an illustration of this point. Meyer (24) found that the minimal concentration of benzamid necessary to produce complete narcosis in tadpoles was $m/500$ at 3° and $m/200$ at 30°, and since the fat solubility of benzamid decreases with rising temperature, he suggested that the higher minimal narcotic concentration at the higher temperature was to be accounted for by the decrease in fat solubility. Experiments of my own with benzamid on *Planaria* (1, pp. 182–190) confirm those of Meyer as regards minimal narcotic concentration in relation to temperature, but with considerably higher concentrations which are lethal within 5 to 10 hours, the relation between susceptibility, concentration and temperature is reversed and death occurs earlier in a given concentration at high than at low temperature, i.e., susceptibility to these higher concentrations increases instead of decreasing with rise in temperature. This susceptibility to slowly lethal concentrations of benzamid shows the same relations as susceptibility to other agents.

Again, it is apparently an established fact that in certain low concentrations alkalies accelerate and acids retard protoplasmic activity, or more specifically oxidations, but in sufficient concentration both are toxic or lethal, and I have found that the regional and individual differences in susceptibility to such concentrations of acids and alkalies are in general similar and like those observed with other agents.

In short, whatever the particular method of action and effect on living protoplasm of an agent in concentrations whose effects are readily reversible, there is a high degree of uniformity in the susceptibility relations of protoplasm to higher, toxic or lethal concentrations of at least a very large number of agents, and these relations are essentially the same as those observed in injurious temperatures and in absence or insufficiency of oxygen.

In general, high susceptibility to concentrations and intensities which kill too rapidly to permit acclimation or the development of tolerance, is associated with a high capacity for acclimation or development of tolerance to a certain range of lower concentration or intensities. Consequently, relative susceptibility can be determined in two ways: first, directly, by the survival time or the degree of inhibition or toxic effect in concentrations or intensities too extreme to permit acclimation or tolerance to develop; second, indirectly, by the capacity of the organism to acclimate or acquire tolerance in a certain range of lower concentrations or intensities. These two ranges of concentration or intensity must be determined experimentally for each agent or condition and for each species of organism, although it is in general true that for closely related species the two ranges of concentration of a given agent do not usually differ very widely.

It is evident from what has been said that the susceptibility relations when acclimation or tolerance are excluded are the opposite of those determined by means of acclimation or tolerance. For example, in all axiate organisms examined thus far, both plants and animals, the susceptibility as determined directly with the higher concentrations decreases primarily from the apical region or the head in the basal or posterior direction, i.e., inhibition, injury or death occur first in the apical or head region and progress in regular manner along the chief axis. In the range of lower concentrations, however, the apical or head region shows the greatest capacity for acclimation or tolerance and in the long run inhibition, injury or death appears first or most distinctly in the basal or posterior region and may be limited to that region or may progress a greater or less distance toward the apical or head region (see, for example, (2), (8), (11), (12), (13), (14)). In view of these facts it is necessary to distinguish between these opposite susceptibility relations. The one depends directly upon the condition of the protoplasm at the moment, the other upon the ability of the protoplasm to alter its condition in the course of time. The two modifications of the method of determining differences in susceptibility have commonly been referred to in the past as the direct and the indirect or acclimation method, since in the one the susceptibility is directly determined, in the other indirectly through the differential capacity for acclimation or tolerance.

I wish particularly to emphasize the fact of the association of these susceptibility relations with certain ranges of concentration or intensity of action of external agents. Investigation of the effects of external agents has been very largely concerned with the dissimilar effects of various low concentrations and intensities, the action of the more highly toxic and lethal concentrations being often regarded as of less interest and importance. Consequently the fact of uniformity of susceptibility relations to a certain range of concentrations or intensities of at least a very large number of external agents and the physiological significance of these relations have not been clearly recognized. This fact is not in conflict with any data concerning the differences in the method of action of different agents or groups of agents within "physiological" concentrations or intensities, but merely indicates that, whatever the method of action in a particular case, a toxic or lethal effect may result with sufficiently high concentration of intensity and that the susceptibility relations to such toxic or lethal effects are, at least to a very high degree, uniform and independent of the particular agent employed, and are not necessarily the same as the susceptibility relations to its action within the limits of rapid reversibility. All the facts indicate that the more or less specific differences in action of different agents on protoplasm are primarily or chiefly associated with reversible or more readily reversible changes, and the uniformity in susceptibility relations with irreversible or less readily reversible changes. If this is true, then the range of concentration or intensity for which this uniformity holds is the range which produces such irreversible or less readily reversible changes in protoplasm.

Concentration or intensity of action of the agent is then of fundamental importance in determining susceptibility relations, and it is only by working with a wide range of concentrations or intensities, from those which kill almost immediately, to those which are non-toxic or merely "physiological" in their action, that any adequate conception of the relations between the protoplasmic system and agents which affect it can be attained. It has been possible to demonstrate more or less completely in various cases that the general susceptibility of protoplasm is related to its metabolic condition, or more specifically to the rate of certain fundamental metabolic reactions, probably primarily the oxidation or certain oxidations. For example, in *Planaria* motor activity increases the direct susceptibility of the worms to cyanides and other agents in toxic concentration (1, pp. 167–170). This experiment I have used extensively as a class experiment. Other forms of stimulation, e.g., section of the body, have also been found to increase both direct susceptibility and CO_2 production (5, p. 437); moreover, direct susceptibility, oxygen consumption and CO_2 production all decrease with advancing physiological age (7). On the basis of these facts alone, these differences in susceptibility might be accounted for by differences in permeability of membranes, but when we find that both direct and indirect susceptibility to temperature and susceptibility to lack of oxygen show the same differences in relation to region of body, physiological age, nutritive condition, etc., as direct and indirect susceptibility to cyanides and other chemical agents, it is evident that we are concerned not merely with permeability as a physical condition but with fundamental activities of protoplasm. Moreover, the fact that the susceptibility relations to low oxygen or lack of oxygen are the same as the direct relations to other agents, indicate very clearly that susceptibility in the sense in which the word is used here is in some way connected with the oxidations or with certain oxidations. And finally, the fact that these susceptibility relations are not specific, but rather essentially identical, showing only slight quantitative differences with a very wide variety of substances and conditions in proper concentration or intensity, indicates that the factor fundamentally concerned is not qualitative and specific, but quantitative.

Bearing all the facts in mind, the simplest and most satisfactory statement at present is that susceptibility is associated with or dependent upon rate of oxidations or of certain oxidations in protoplasm. While I have made this statement repeatedly, I have likewise endeavored to point out that it must not be interpreted as meaning that all agents and conditions act directly or in any specific way upon the oxidative reactions, or even that all act in the same way on protoplasm. But whether a particular agent enters chemically into the oxidative reaction, acts as a reducing agent, affects the oxidizing enzymes or alters the aggregate condition of the protoplasm colloids in some way, susceptibility to that agent within certain limits of concentration may still be dependent on and a measure of the relative rates of oxidation in different body-regions and in different conditions of age, nutrition, activity, etc. This is all that such a statement can mean at present, though in future, as we learn more of the action of each particular agent on protoplasm, we shall doubtless be able to state more definitely the nature of the relation between the action of each agent and the rate of oxidation. At present, however, it is scarcely possible to say more than that the rate of oxidation is dependent upon a complex of factors in living protoplasm and that each such factor may play a rôle both as an index and a determiner of oxidation rate.

The biochemist has frequently said that such expressions as "rate of metabolism" and "rate of oxidation" have no value or meaning.

From a certain biochemical viewpoint which conceives each particular chemical reaction as a discrete entity, this statement is unquestionably correct. But it is just as certain that from a certain physiological viewpoint these expressions possess a very real significance and are at present necessary for statement of the experimental data. The general relation between susceptibility and oxidation rate is an experimental fact, and the statement of this relation is merely the first step toward its analysis. The probability that if we knew more about the matter we might be able to state the relation in more definite terms for each particular case, does not lessen the value of the general statement as a starting point. Living protoplasm is a system of physiologically correlated and interdependent changes and conditions, not a mosaic of wholly independent reactions. When we examine this system in its chemical aspects, the oxidations appear as highly significant or fundamental factors, while in another aspect the active mass of enzymes, colloid aggregation or permeability of membranes may be regarded as fundamental. But all such factors are unquestionably more or less closely correlated and it is the same system in all cases. With certain limitations we can use the rate of oxidation in such a protoplasmic system as a measure of its physiological or vital condition, and, so far as this is possible, the conception is justified. The relation between susceptibility and rate of oxidation is simply this relation between rate of oxidation and physiological condition stated in terms of the action of external agents. Like any other general statement of relation it is subject to modification and limitation and may give place to more definite statements as experiment advances our knowledge, but as far as it goes, it is a statement of fact.

This relation may be stated in the following terms: The direct susceptibility, i.e., susceptibility to the agents employed in sufficient concentration to prevent acclimation or acquirement of tolerance, but not immediately lethal, varies in general directly, though not necessarily proportionally with the rate of oxidation (1), (4), (7, chap. iii). The indirect susceptibility associated with ability to acclimate or acquire tolerance to a certain range of low concentrations and intensities of the agents employed, varies inversely though not necessarily proportionally with rate of oxidation.

To my mind these susceptibility relations are simply one expression of the fact that living protoplasm is a system of interdependent and correlated conditions and changes, in which dynamic equilibration is continuously going on and new disturbances are continuously occurring. Since regional and individual differences in the condition of this system exist, there must also exist regional and individual differences in susceptibility to all agents which alter to a sufficient degree any of the correlated factors constituting the system. Moreover, these differences in susceptibility must show a certain definite and in general uniform relation to certain fundamental factors in the system. Such, I believe, is the relation between susceptibility and oxidation rate; not in any sense specific, not necessarily direct, undoubtedly to be stated in different terms in different cases, and meaning merely that rate of oxidation is associated with and dependent on various factors in the protoplasmic system which are susceptible to alteration by external agents.

No claim is made for universality of the relations as stated, though they have been experimentally demonstrated for a large number of agents and organisms (pp. 372 to 373). It remains to determine their limitations, their nature in particular cases and their value as a method of investigation. As regards the latter point, their value as a method for attacking various problems has already been abundantly demonstrated. The susceptibility method, checked in various ways, has served not only for demonstrating physiological axes in plants (11), (13), (14), protozoa (3) and various other animals from nearly all the great groups, but it has made possible the control and modification of development and a rational interpretation of various types of teratology (12), (16) and experimental data to be published later will show that it has given us a basis for interpretation of galvanotactic reactions and bio-electric currents. Moreover, it has been an important factor in the development of a conception of physiological polarity, axiation and integration which has received experimental support and confirmation from many different sources (8). Practically it has amply justified itself.

In work with this susceptibility method the cyanides have proved to be very useful agents, particularly in the direct determination of susceptibility, because of their high toxicity and the very slow and slight acclimation to their action. Moreover, various lines of evidence which are discussed by Doctor Hyman in the paper appearing in this number of this Journal, show very clearly that the cyanides do act in some way on the rate of oxidation. As regards the general susceptibility relations, the differences between cyanides and other agents are merely quantitative. Salts of the heavy metals, e.g., $HgCl_2$, $CuSO_4$, etc., can be used in even greater dilution than the cyanides for susceptibility determination and the same regional and individual differences appear in survival time, rate of development and form and proportion as with cyanides, the chief difference observed being that acclimation or tolerance to the heavy metal salts develops somewhat more rapidly and to a greater degree than to cyanides.

Cyanides are not used as "specific" agents for determination of susceptibility, as certain critics of the method have apparently believed, but merely as convenient agents, because they act in low concentration and so permit the exclusion of osmotic or other effects associated with high concentration and because the very slow and slight acclimation makes it possible to distinguish sharply between direct and indirect or acclimation susceptibility. Whatever the nature of the relation between the toxicity of cyanides and the protoplasmic oxidations, much evidence has accumulated to show that cyanides decrease oxidation in some way, although in some cases a primary stimulation occurs with extremely low concentration. For all these reasons the cyanides have been much used in the work on susceptibility, but essentially the same results may be obtained with many other agents.

The most extensive studies on susceptibility in relation to different body regions, to differences in physiological age, nutritive conditions, temperature, etc., have been made with the flatworm *Planaria dorotocephala* as material (1), (2), (4), (5), (7), (10). Since the cyanides have been much used in this work and for other forms as well, it seems desirable, even though the results obtained have already been checked by CO_2 estimation with the Tashiro biometer (1), (7, chap. iii) and in various other ways, to present additional data concerning the effect of cyanides on oxidation in this species. From what we already know, there can be little doubt that cyanides decrease oxidation at least very generally in organisms and the evidence already at hand indicates very clearly that they act in this way on *Planaria*. Nevertheless, since so much work has been done with *Planaria* as material and the cyanides as agents it seems worth while to make the demonstration as complete as possible. Such a demonstration serves to throw some light not only on the relation between susceptibility to cyanides and rate of oxidation, but on the whole problem of susceptibility to agents in general.

Dr. Hyman's paper presents the data on the effect of cyanide on oxygen consumption in *Planaria*, as determined by the Winkler method, and the following sections of the present paper give some supplementary data on the effect of cyanide on CO_2 production, estimated colorimetrically, and on susceptibility to lack of oxygen, determined by survival time.

THE EFFECT OF CYANIDE ON THE PRODUCTION OF CARBON DIOXIDE

In these determinations the colorimetric method with phenolsulphonephthalein as indicator was employed, the Hynson, Westcott & Dunning set of colors in buffer solutions being used as the standard for comparison. For each experiment worms from the same stock, of the same size and nutritive conditions are used in two lots of as nearly as possible equal weights. In nearly all experiments the heads are removed before weighing in order to prevent as far as possible motor activity, which in animals with heads may be sufficient to increase the CO_2 production to a considerable extent. It has been determined that removal of the heads has no immediate effect on CO_2 production except a slight temporary increase during the first few hours after decapitation. The heads are of course regenerated, and after two days or more there is an increase in CO_2 production connected with the development of the embryonic tissue which gives rise to the new head, but within the first forty-eight hours after section this is very slight or inappreciable as compared with the total and the animals were in most cases used within this period. Moreover, since the number of worms in the two lots to be compared is equal or nearly equal, the number of regenerating regions is the same or nearly the same in both, and the differences in CO_2 production from this source are negligible.

The animals are weighed dry, i.e., they are placed on filter paper to remove excess water before weighing and then transferred on the blade of a small lancet to a small glass container already balanced and standing on the balance pan. Weighing is done as rapidly as possible for injury results from exposure to the air for more than one or two minutes. As soon as weighed the animals are returned to water, and then in some cases a preliminary comparison of CO_2 production in the two lots is made before subjecting one of them to the action of KNC.

For the colorimetric determination of CO_2 pyrex glass tubes of 5 to 6 cc. volume and of the same diameter as the standard tubes are used, the worms being placed in these tubes in equal volume of phenolsulphonephthalein solution made up to give the same density of color as the colorimeter tubes. The open end of the tube is first closed with a small plug of thoroughly washed absorbent cotton soaked in the same indicator solution, air bubbles being excluded, and is then sealed with melted paraffine. This method of closure has been found preferable for these animals to the rubber tubing and clamp used by Haas (25), because even the headless animals may creep about to some extent during the first few moments of the experiment, and if rubber tubing and clamp are used they tend to come to rest in the rubber tubing, where the light intensity is least. There they are out of sight and differences in motor activity cannot be observed, moreover they are often crushed or lost in removal. With the cotton-paraffine closure the cotton serves for the usual period of an experiment as an inner seal.

The CO_2 production is determined in terms of pH, the minus logarithm of the hydrogen ion concentration, by matching at intervals the tubes containing the worms with the standard tubes of known pH. That the animals do not produce any non-volatile acids in appreciable quantities is demonstrated by the fact that after change in color of the indicator by the worms, shaking with air restores the original color, and the probability that any other volatile acid than carbonic acid is produced is negligible.

The preliminary pH determination usually extends over two or three hours with half-hourly or hourly readings of pH. At the end of this time the animals are removed from the tubes, one lot being placed in water, the other in KNC of the concentration used in that particular experiment. The animals remain in KNC for various lengths of time, but before death and disintegration of any part begin (in a few cases with the longer periods in KNC very slight traces of disintegration were observed), they are returned again to water, washed in several changes of water and allowed to remain from 5 to 30 minutes before being placed in the indicator solution. After this time both the cyanide lot and the control are again placed in clean tubes with fresh indicator solution, the tubes sealed and the pH determined half-hourly or hourly or at longer intervals for several hours. With some lots the experiment was concluded at this point, but with others observations were made on recovery, the two lots being again returned to water and their CO_2 production being again compared after 24 or 48 hours. In many experiments then there are three comparative estimations of CO_2 production, first preliminary, second immediately after KNC, third during or after recovery.

The chief disadvantage of this method is that the CO_2 estimation is made after removal of the animals from cyanide rather than in its presence. The KNC solution is of course alkaline and its alkalinity changes more or less on standing so that it is difficult to bring it to the same pH as the water used in the control. The effect of cyanide determined in this way is then merely the effect that remains after return of the animals to water. The animals begin to recover during the CO_2 estimation, and partial recovery sometimes appears in the later stages of the estimation. This method serves merely to demonstrate that CO_2 production is affected by cyanides, but does not give quantitative data. The determinations of the effect of cyanides on oxygen consumption in Doctor Hyman's paper give much more striking results than those on CO_2 production, because the oxygen consumption is determined in the presence of cyanide, not merely after its action.

The water used in all experiments is a well water, the same in which the stocks of worms are kept, the tap water being unavailable because of chlorination. When this water is first pumped $pH = 7.6$, but on standing a small amount of CO_2 is given off and the pH rises to 7.8 or even to 8.0. This accounts for the fact that in the following tables the pH at the beginning of different experiments is different. Since the relative CO_2 production of cyanide and control lots is of interest here rather than the absolute CO_2 production, the pH at the beginning of the experiment need not always be the same in different experiments, but is always the same for experimental and control lots in any one experiment. The results are given in terms of pH, the decrease in pH representing the increase in hydrogen ion concentration resulting from CO_2 production in the respiration of the animals. In the pH data as given in the table the figures in the first decimal place are obtained directly by matching with the standard tubes, while those of the second place are based on estimation from the two standard tubes between which the color of the experimental tube lies.

The chief data are presented in tables 1 to 4. The first four vertical columns in tables 1 and 4, the first three columns in tables 2 and 3 give the general data of each series, series number, length in milligrams of animals used where the table includes worms of different length, the number of worms in each lot and the weights of each lot in fractions of a gram. The remaining columns have to do with the data of each particular experiment. In most series the data are divided into three groups as indicated in the column headed experimental conditions: first, preliminary, where both lots A and B are in water; second, KNC, where lot A has been in KNC of the concentration and for the time indicated; third, recovery, in which the animals of lot A have been in water a certain number of hours as indicated in each experiment since treatment with KNC. In a few series, e.g., series 680 in table 1, no observations were made on recovery, while in series 667 II in table 2 two different periods of recovery are recorded, but no preliminary observations were made. The columns giving the pH represent half-hour or hour intervals, but the actual intervals between observations differ as indicated in different series.

The important point in each series is the difference in pH which appears during two to four hours between the cyanide lot A after exposure to KNC and the control lot B, as compared with the difference between these two lots in the preliminary and the recovery determinations. It will be observed that in every series the decrease in pH in lot A, the lot exposed to KNC *is less rapid after exposure as compared with that in lot B* than in either the preliminary or the recovery determinations. This difference is not due to difference in weight in any case, for not only are the differences in weight between the two

TABLE 1

Well-fed worms 16 to 25 mm KNC *m/1000*

SERIES	LENGTH	NUMBER OF WORMS	WEIGHTS	EXPERIMENTAL CONDITIONS		pH AT HALF-HOUR INTERVALS					
	mm.		*grams*			0	½	1	1½	2	3
680 A	25	5	0.1064	Preliminary	A. water	7.6	7.5	7.33	7.25	7.2	
680 B	25	5	0.1043		B. water	7.6	7.5	7.33	7.25	7.2	
				KNC	A. KNC 3 hours	7.6	7.43			7.27	
					B. water	7.6	7.4			7.22	
696 A	16	8	0.08	Preliminary	A. water	7.7	7.57	7.5	7.4	7.3	
696 B	16	8	0.0757		B. water	7.7	7.6	7.55	7.43	7.33	
				KNC	A. KNC 3 hours	7.75	7.6	7.5		7.35	7.25
					B. water	7.75	7.53	7.4		7.27	7.17
				Recovery 48 hours	A. water	7.77		7.43		7.25	
					B. water	7.77		7.43		7.25	
692 A	16–18	8	0.0865	Preliminary	A. water	7.77	7.6	7.53			7.2
692 B	16–18	8	0.0814		B. water	7.77	7.63	7.55			7.23
				KNC	A. KNC 5½ hours	7.6	7.5			7.3	
					B. water	7.6	7.43			7.23	
				Recovery 43½ hours	A. water	7.7	7.55	7.4	7.35		7.13
					B. water	7.7	7.55	7.43	7.37		7.15

TABLE 2

Well-fed worms 8 to 10 mm. KNC *m/1000*

SERIES	NUMBER OF WORMS	WEIGHTS	EXPERIMENTAL CONDITIONS	pH AT HOURS							
				0	½	1	1½	2	2½	3	4
		grams									
			KNC: A. KNC ½ hour	7.75		7.73				7.7	7.63
			KNC: B. water	7.75		7.7				7.6	7.5
667 II A	4	0.0131	Recovery 22 hours: A. water	7.75				7.7			7.65
667 II B	3	0.0138	Recovery 22 hours: B. water	7.75				7.67			7.55
			Recovery 72 hours: A. water	7.77		7.75					7.63
			Recovery 72 hours: B. water	7.77		7.73					7.6
			Preliminary: A. water	7.9	7.8	7.73	7.63	7.53			
			Preliminary: B. water	7.9	7.8	7.73	7.63	7.53			
679 A	11	0.0364									
679 B	10	0.0364									
			KNC: A. KNC 1 hour	7.9				7.58		7.47	
			KNC: B. water	7.9				7.53		7.4	
			Preliminary: A. water	7.6	7.55	7.47	7.43	7.38			
			Preliminary: B. water	7.6	7.55	7.47	7.43	7.38			
681 A	11	0.0333	KNC: A. KNC 1 hour	7.6	7.57	7.53	7.48	7.43		7.35	
681 B	10	0.0315	KNC: B. water	7.6	7.55	7.5	7.43	7.37		7.3	
			Recovery 42 hours: A. water	7.7		7.65	7.5	7.4			
			Recovery 42 hours: B. water	7.7		7.6	7.5	7.4			
			Preliminary: A. water	7.65	7.55	7.47	7.4				
			Preliminary: B. water	7.65	7.55	7.45	7.4				
683 A	10	0.0433	KNC: A. KNC 2 hours	7.7	7.67	7.58	7.5			7.4	
683 B	10	0.0415	KNC: B. water	7.7	7.6	7.5	7.4			7.3	
			Recovery 25 hours: A. water	7.77	7.67				7.4		
			Recovery 25 hours: B. water	7.77	7.7				7.42		

lots A and B of a pair slight, but in all cases the preliminary determinations show almost the same or exactly the same rate of change in pH in A and B, and in all series except 667 II in table 2 and 686 in table 3, where there is any difference in weight between lots A and B, A is the heavier and should therefore produce more CO_2 than B. After a certain length of time in KNC, however, A produces less CO_2 than B. Differences in motor activities do not account for this difference, for in most cases the headless animals showed little or no motor activity during the determinations, and all results in which differences in motor activity were sufficient to have constituted a possible factor in the pH differences are excluded from the tables. There is no escape from the conclusion that the difference in rate of change of pH after KNC is due to a difference in rate of CO_2 production which does not

TABLE 3

Worms starved 51 to 53 days: 8 to 10 mm. KNC *m/1000*

SERIES	NUMBER OF WORMS	WEIGHTS	EXPERIMENTAL CONDITIONS	pH AT HOURS								
				0	½	1	1½	2	2½	3	3½	4
		grams										
686 A B	8 8	0.0164 0.0168	Preliminary A. water	7.8		7.75	7.7			7.6		
			Preliminary B. water	7.8		7.75	7.7			7.62		
			KNC A. KNC 2 hours	7.6				7.53		7.47		
			KNC B. water	7.6				7.5		7.43		
687 A B	9 8	0.0451 0.0444	Preliminary A. water	7.8	7.7	7.6	7.55		7.4			
			Preliminary B. water	7.8	7.72	7.62	7.58		7.43			
			KNC A. KNC 1 hour	7.6			7.43		7.37		7.3	
			KNC B. water	7.6			7.4		7.33		7.23	
			Recovery 19 hours A. water	7.77		7.62		7.4				7.3
			Recovery 19 hours B. water	7.77		7.62		7.42				7.3
684 A B	8 8	0.0333 0.0332	Preliminary A. water	7.7		7.6	7.55	7.47				
			Preliminary B. water	7.7		7.6	7.55	7.47				
			KNC A. KNC 1½ hour	7.77		7.7				7.52		7.37
			KNC B. water	7.77		7.65				7.4		7.27
			Recovery 43 hours A. water	7.67		7.6		7.38				7.3
			Recovery 43 hours B. water	7.67		7.6		7.4				7.33

TABLE 4

Well-fed worms 16 to 25 mm. KNC *various concentrations*

SERIES	LENGTH	NUMBER OF WORMS	WEIGHTS	EXPERIMENTAL CONDITIONS		pH AT HOURS							
						0	½	1	1½	2	2½	3	4
	mm.		*grams*										
682 A	25	5	0.1187	Prelimi-nary	A. water	7.65	7.45	7.3	7.2	7.15			
682 B	25	5	0.1152		B. water	7.65	7.47	7.32	7.22	7.17			
				KNC *m*/500	A. KNC 2¾ hours	7.7	7.57	7.4					
					B. water	7.7	7.52	7.32					
				Recovery 24 hours	A.water	7.77	7.5				7.2		
					B.water	7.77	7.5				7.2		
689 A	25	6	0.1304	Prelimi-nary	A. water	7.7	7.57	7.37	7.3				
689 B	25	6	0.1285		B. water	7.7	7.57	7.35	7.28				
				KNC *m*/2000	A. KNC 5 hrs.	7.75	7.5				7.23		
					B. water	7.75	7.45				7.17		
691 A	18–20	7	0.086	Prelimi-nary	A. water	7.75	7.53	7.38					
691 B	18–20	6	0.0846		B. water	7.75	7.55	7.4					
				KNC *m*/10000	A. KNC 22½ hrs.	7.6	7.4	7.33					6.87
					B. water	7.6	7.37	7.27					6.8
				Recovery 45 hours	A.water	7.8	7.6	7.4	7.33	7.27			
					B.water	7.8	7.6	7.4	7.32	7.27			
688 A	25	9	0.2253	Prelimi-nary	A. water	7.6	7.4		7.2				
688 B	25	9	0.2182		B. water	7.6	7.43		7.2				
				KNC *m*/20000	A. KNC 61½ hrs.	7.7	7.5	7.25					
					B. water	7.7	7.47	7.2					
693 A	16–18	9	0.094	Prelimi-nary	A. water	7.77	7.67				7.3		
693 B	16–18	8	0.0923		B. water	7.77	7.67				7.32		
				KNC *m*/25000	A. KNC 21½ hrs. hours	7.6	7.35			7.17			
					B. water	7.6	7.32			7.1			
				Recovery 24 hours	A.water	7.7	7.6	7.45				7.23	
					B.water	7.7	7.63	7.5				7.27	

exist in the preliminary nor in the recovery determinations to any such degree. Carbon dioxide production is unquestionably decreased by KNC.

In all series of tables 1, 2 and 3, KNC $m/1000$ was used. this being the concentration commonly employed for direct susceptibility determinations, but table 4 shows the effects of various concentrations ranging from $m/500$ to $m/25000$ with various periods of exposure.

A comparison of the preliminary, the recovery and the cyanide determinations shows in some cases that the absolute decrease in pH is more rapid in both A and B in the cyanide than in the preliminary or the recovery determinations. Series 693, table 4 is the most extreme case of this sort. Differences in temperature constitute the chief factor in determining differences of this kind. Most of this work was done during the winter months and the range of variation in the laboratory temperature was about 5°C. This range of variation may affect CO_2 production in different determinations, but in any one determination temperature conditions for the two lots are of course the same. Stimulation resulting from the necessary handling may also be concerned in some cases. In the KNC determination both the cyanide lot and the control are handled as nearly as possible in the same way, the control being washed as frequently as the KNC lot. These washings, together with the transfer to the tubes in pipettes, may cause a considerable degree of excitation which does not necessarily appear in increased motor activity, but does increase the rate of pH change during the first half-hour or so.

A considerable number of series not included in the tables was run without preliminary pH determination, but in all such cases the decrease in pH was less rapid in the KNC lot than in the control. Among all determinations made, only one, with KNC $m/10000$. showed a slightly higher rate of pH decrease in the KNC lot than in the control. This low concentration of KNC has but little inhibiting effect, except when its action is continued for a day or more, and in this particular case the KNC lot showed a greater motor activity than the control.

All determinations of table 1 were made with large, physiologically old worms, 16 to 25 mm. in length, while in those of table 2 the worms were considerably younger, only 8 to 10 mm. in length. A comparison of tables 1 and 2 shows that the smaller, physiologically younger worms are more susceptible to KNC than the larger, older animals, although no exact expression of this difference is obtainable from the data. The tables show merely that the retardation in the rate of change of pH in KNC $m/1000$ in the younger animals is as great as, or greater than that in the older, with shorter times of exposure to KNC and although the total weights of the lots of young worms are only one-half to one-tenth those of the old. The differences in susceptibility indicated are in complete agreement with those previously observed in relation to physiological age (4), (7). Moreover, the CO_2 production, as indicated by pH, of the worms of table 3 which have been starved about 50 days is much more nearly like that of the young animals of table 2 than that of the older animals of table 1, a fact which also agrees, as might be expected, with the results of the direct susceptibility method.

In table 4, where the results for very different concentrations are given, only a few characteristic records are selected from among a larger number obtained. It is evident that with decrease in concentration a very great increase in time of exposure is possible without death. In the concentrations used in series 688 and 693, viz., $m/20000$ and $m/25000$, there is probably some degree of acclimation. The effect of $m/20000$ is no greater after 61½ hours in series 688 than of $m/25000$ after 21¾ hours in series 693, and I have found in other experiments that some degree of acclimation may occur in these concentrations.

In the recovery determinations it was not infrequently observed that the cyanide lot A showed a higher CO_2 production in relation to the control than in the preliminary determinations. Series 683, table 2 and 693, table 4, show this condition. These results suggest the occurrence of a period of supernormal CO_2 production in the course of recovery, but further data are necessary to establish this point.

The attempt was made with very low concentrations and short periods of exposure to determine whether a stimulating effect, such as has been observed with low concentrations of cyanides in various other cases, could be detected. Animals in KNC $m/100,000$ for ¼, ½ or 1 hour and those in $m/50000$ for ¼, ½ or 1½ hour showed no appreciable effect, either of stimulation or depression. After 3 hours in KNC $m/50000$ or ¾ hour in $m/20000$ there was very slight but distinct decrease in CO_2 production, as indicated by pH, extending over at least 2 hours. In these, as in the series with higher concentrations, the pH was determined after removal from the cyanide.

In another group of experiments the cyanide was made up in the indicator solution and brought as nearly as possible to the same pH as the water by the addition of HCl $m/1000$ and the CO_2 production in this solution was compared with that of the control in water and with that of a preliminary determination. In these experiments where CO_2 production was estimated in the presence of KNC concentrations down to *m*/250000 showed apparently a distinct inhibiting action and even in *m*/500000 some slight inhibition seemed to occur. These data are perhaps not very conclusive because of the difficulty of bringing the KNC solution to the same pH as the water and because of the possibility of change in alkalinity in the solution itself. No indication of stimulation was observed in any case.

THE EFFECT OF CYANIDE ON SUSCEPTIBILITY TO LACK OF OXYGEN

For determining susceptibility to lack of oxygen water from which the oxygen has been largely removed by boiling or by other means is used. The well water used in the laboratory has so low an oxygen content before aeration, analysis giving only a trace of oxygen, that it can be used directly for this purpose. Susceptibility to lack of oxygen is determined by observing the survival times of animals in small volumes of low oxygen water, sealed so as to avoid entrance of air. In my experiments the same pyrex tubes were used as for the CO_2 experiments, but with only about 2 cc. of water. Since relative rather than absolute susceptibility is of interest for present purposes, two lots of individuals to be compared, consisting of three or four individuals each, may be placed in the same tube and so be under identical conditions as regards oxygen and CO_2. If the animals of the two lots are not readily distinguishable by size or other external characteristics, one lot may be marked, e.g., by cutting off a bit of the posterior end. This slight injury has no appreciable effect on the total susceptibility, but if we mark the lot expected to show the lower susceptibility of two to be compared, any stimulation which might result will only decrease the difference in susceptibility between this and the other lot.

The method of introducing the two lots into the same tube makes weighing unnecessary, for it makes no difference whether the two lots are of equal weight or not. It is of course also possible to compare two lots of equal weight in different tubes with equal volumes of water of the same oxygen content, but the possibilities of error are greater with this method.

In order to make certain that it is lack of oxygen and not accumulation of CO_2 that kills, sufficient NaOH may be added to make NaOH *m*/1000. This concentration is well below the toxic limit and prevents too great acidity by the CO_2 produced. By adding also a little phenolsulphonephthalein to such a solution it is found that the animals almost always die from lack of oxygen while the solution is still alkaline, often while the alkalinity is pH 8.2 or more, i.e., near or beyond the limit of this particular indicator on the alkaline side. It is perfectly clear that death in such cases results from lack of oxygen, not from CO_2. Moreover, by introducing more or less oxygen into such a solution life may be prolonged as desired or recovery may be brought about after partial death and disintegration.

The first visible effect of lack of oxygen in *Planaria* is an increased negative geotaxis, then follows a decrease and loss, beginning in the head region, of the ability to attach to the substratum and creep and of muscular activity in general. Sooner or later the animals become completely inactive and lie more or less completely relaxed on the bottom, and death and disintegration follow. These changes occur first in the head region and in the posterior zoöids and from the head they progress posteriorly along the first zoöid. In other words, the gradient of susceptibility to lack of oxygen is the same as that to the higher concentrations of KNC and other agents (1), (2), (3), (4), (6), (9), (17), (19). Disintegration of the head and the posterior zoöids may occur while other regions are still alive and capable of recovery. In *Planaria* disintegration follows death almost at once or perhaps may precede death of many of the cells, and may be used as a convenient criterion of death.

There can be no doubt that this susceptibility to lack of oxygen is a more or less exact measure of oxidative metabolism and the fact, to which attention has already been called, that it shows the same regional and individual differences as direct susceptibility to other agents is significant evidence for the general relation between susceptibility and oxidative metabolism.

In a part of my experiments on the effect of cyanide on susceptibility to lack of oxygen the control and the cyanide lot after exposure to cyanide were placed in the same tube in low oxygen water. In others lots of equal weight were placed in different tubes in equal volumes of water, and in still others KNC was added to the low oxygen water in one tube and the susceptibility in this solution compared with that of a lot of equal weight in water. Only the low concentrations of KNC can be used in this way because the higher concentrations kill the animals before they are affected by lack of oxygen. Since animals affected by cyanide to any great degree show less motor activity than normal animals, the heads of all worms were removed.

The data for six series are given in table 5 in general form, without details of the progress of death and disintegration. Other series are essentially similar with similar results to some one of these six.

In the first three series of the table the animals of the experimental lot were placed in KNC for three or four hours and removed to water before the susceptibility determination, as indicated by "preceding" in the fourth column of the table, and KNC and control lots were placed in the same tube. In the last three, lots of equal weight were used and the KNC was added to the low oxygen water of the one tube of the pair and so was present "during" the susceptibility determination, KNC lot and control being in separate tubes.

In every series except 672 the susceptibility of the experimental (KNC) lot is distinctly greater. with the higher concentrations much greater than that of the control. In series 672 the susceptibility of the control was at first slightly greater, but in later stages all individuals of the cyanide lot were distinctly more susceptible. Whether this unusual result was due to the low concentration of the KNC or to other incidental factors could not be determined.

TABLE 5

Effect of KNC on susceptibility to lack of oxygen

SERIES	LENGTH	CONCENTRATION OF KNC	EXPERIMENTAL LOT IN KNC	SUSCEPTIBILITY
	mm.			
671	25	*m*/1000	3 hours preceding	Experimental lot > Control
670	25	*m*/1000	4 hours preceding	Experimental lot > Control
673	25	*m*/1000	4 hours preceding	Experimental lot > Control
675	25	*m*/10000	During	Esperimental lot > Control
676	8–10	*m*/20000	During	Experimental lot > Control
672	25	*m*/50000	During	Experimental lot ≶ Control

Animals in KNC, or that have been in KNC, are then more susceptible to lack of oxygen than normal animals. Since we know from other experiments that increase in rate of oxidation increases susceptibility to lack of oxygen, these results may appear at first glance to indicate that KNC increases the rate of oxidation. Such a conclusion, however, is directly opposed to all that we know in general concerning the action of the higher concentrations of cyanides on protoplasm. Not only Doctor Hyman's results on oxygen consumption and my own on CO_2 production, but many other experimental facts indicate very clearly that the cyanides in some way decrease oxidation. How then shall we interpret these data on susceptibility to lack of oxygen?

In the light of all the known facts there is every reason to believe that living protoplasm subjected to the action of cyanides is in much the same condition as when subjected to lack of oxygen, i.e., the cyanides prevent or retard in some way the oxidative reactions or certain of them in such a way that their effect is additive to that of lack of oxygen. If this is the case, the lack of oxygen in my experiments continues or is added to the action begun by KNC, consequently the animals subjected to cyanides are more susceptible to lack of oxygen than the controls.

This fact, that cyanides increase susceptibility to lack of oxygen, constitutes, I believe, still further evidence in support of the conclusion that cyanides inhibit oxidation in some way and it agrees with the conclusion reached by Geppert (26) in suggesting that this action consists in preventing the tissues from obtaining or from utilizing oxygen which is present. Such an effect may be due to destruction or inactivation of oxidizing enzymes or to action as a reducing agent or to some other unknown method of action.

CONCLUSION AND SUMMARY

The results presented above are far less striking than Doctor Hyman's data on the effect of cyanides on oxygen consumption, and are to be regarded as merely supplementary to them. Doctor Hyman has measured oxygen consumption in the presence of cyanides while I have been concerned almost wholly with what remains of the effect after return of the animals to water. While all the data agree as regards the effects of KNC, they permit only certain general conclusions concerning quantitative differences in susceptibility, but these so far as they go, are in complete agreement with the conclusions drawn from experiments of other sorts.

The points of chief importance are summarized as follows:

1. A colorimetric method with phenolsulphonephthalein as indicator can be readily used for the comparative estimation of CO_2 production in terms of pH in *Planaria dorotocephala*. Differences in CO_2 production with physiological age, nutritive condition, etc., are easily demonstrated by this method.

2. KNC in concentrations ranging from $m/500$ to $m/25000$ and with periods of action from ½ to 61½ hours decreases CO_2 production in *Planaria dorotocephala*.

3. If the exposure to KNC is not too long, gradual recovery in CO_2 production occurs, apparently with a supernormal stage near the end of the recovery period.

4. Susceptibility to lack of oxygen in *Planaria* shows in general the same regional and individual differences as susceptibility to cyanides and other toxic agents. KNC increases susceptibility to lack of oxygen, i.e., an animal which has been in cyanide dies earlier from lack of oxygen than a normal control animal.

5. Since cyanides decrease both oxygen consumption and CO_2 production in *Planaria*, the increase by cyanide in susceptibility to lack of oxygen can mean only that cyanide and lack of oxygen are to some extent additive in their action on living protoplasm, i.e., their action must be in certain respects identical or similar in character.

BIBLIOGRAPHY

(1) CHILD: Journ. Exper. Zoöl., 1913, xiv, 153.
(2) CHILD: Arch. f. Entwicklungsmech., 1913, xxxvii, 108.
(3) CHILD: Biol. Bull., 1914, xxvi, 36.
(4) CHILD: Arch. f. Entwicklungsmech., 1914, xxxviii, 418.
(5) CHILD: Journ. Exper. Zoöl., 1914, xvi, 413.
(6) CHILD: This Journal, 1915, xxxvii, 203.
(7) CHILD: Senescence and rejuvenescence, Chicago, 1915.
(8) CHILD: Individuality in organisms, Chicago, 1915.
(9) CHILD: Biol. Bull., 1916, xxx, 391.
(10) CHILD: Journ. Exper. Zoöl., 1916, xxi, 101.
(11) CHILD: Botan. Gaz., 1916, lxii, 89.
(12) CHILD: Journ. Morphol., 1916, xxviii, 65.
(13) CHILD: Biol. Bull., 1916, xxxi, 419.
(14) CHILD: Biol. Bull. 1917, xxxii, 175.
(15) CHILD: This Journal, 1917, xliii, 87.
(16) CHILD: Journ. Morphol., 1917, xxx, 1.
(17) HYMAN: Journ. Exper. Zoöl., 1916, xx, 99.
(18) HYMAN: This Journal, 1916, xl, 238.
(19) HYMAN: Journ. Exper. Zoöl., 1917, xxiv, 55.
(20) LILLIE: This Journal, 1914, xxxiv, 414.
(21) LILLIE: This Journal, 1915, xxxvii, 348.
(22) LILLIE: Biol. Bull., 1916, xxx, 311.
(23) OSTERHOUT: Proc. Amer. Phil. Soc., 1916, lv, 533.
(24) MEYER: Arch. f. exper. Path. u. Pharm., 1901, xlvi, 338.
(25) HAAS: Sci., 1916, xliv, 105.
(26) GEPPERT: Zeitschr. f. klin. Med., 1889, xv, 208, 307.
(27) HYMAN: Sci., 1918, xlviii, 518.

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FURTHER OBSERVATIONS SHOWING THAT EPINEPHRIN FROM THE ADRENALS IS NOT INDISPENSABLE

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We showed in a previous paper (1) that cats live indefinitely in good health after excision of one adrenal and section of the nerves of the other, an operation which either abolishes the output of epinephrin from that adrenal or reduces it to a small fraction of the normal. It is well known that a certain proportion of rabbits survive double adrenalectomy, in our experience something like 20 per cent (2). Whether this is due in all cases to the presence of accessory adrenals or not, it is clear that in rabbits also the epinephrin secretion of the adrenals is not indispensable since accessory adrenals consist only of cortical tissue. In this investigation we have extended the observations to dogs and monkeys, with the same result; neither the length of life nor the health of the animals could be shown to be in any way affected by removal of one adrenal and section of the nerves of the other. Some protocols with samples of tracings used in assaying the epinephrin in the adrenal vein blood at the time the animals were sacrificed are reproduced.

CONDENSED PROTOCOL

Dog 241. Male. Weight 6.92 kgm.

October 21, 1918. Right adrenal excised. It weighed 0.55 gram and contained 0.47 mgm. epinephrin. Nerves of left adrenal cut (major and minor splanchnics, and all twigs seen going to the adrenal).

November 11, 1918. Condition excellent, weight 7.5 kgm.

Under morphine and ether, obtained a specimen of blood from the jugular vein. Then made cava pocket in usual way, tying abdominal aorta but not the coeliac and mesenteric arteries.

Collected the following specimens of adrenal blood:
1st specimen, 2.1 grams in 1½ minutes (1.4 grams per minute)
2nd specimen, 6.85 grams in 5 minutes (1.37 grams per minute)
3rd specimen, 6.0 grams in 5 minutes (1.2 grams per minute)
4th specimen, 3.3 grams in 4 minutes (0.8 gram per minute)

Now obtained additional indifferent blood from the jugular and a sample from the abdominal aorta. The left adrenal weighed 0.562 gram and contained 0.66 mgm. epinephrin.

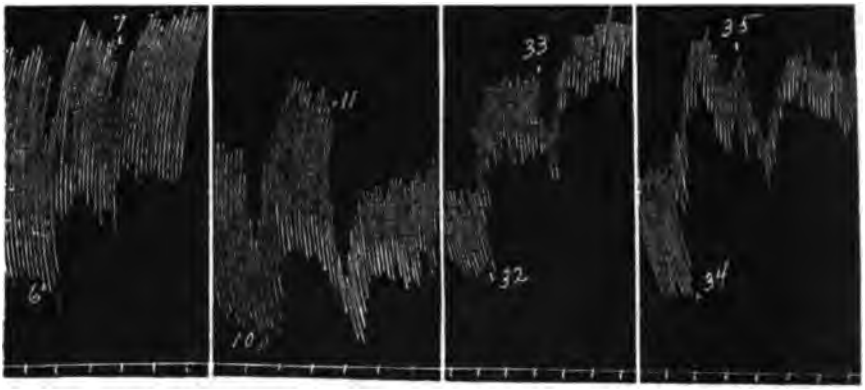

Fig. 1. Intestine tracings. Bloods from dog 241. At *6*, Ringer was replaced by jugular blood and this at *7* by the 4th adrenal specimen; at *10* Ringer was replaced by jugular blood, and this at *11* by jugular blood to which was added adrenalin to make a concentration of 1: 62,500,000. The bloods were diluted with three volumes Ringer (the adrenalin blood after adding the adrenalin); at *32* Ringer was replaced by jugular blood and this at *33* by the 3rd adrenal specimen; at *34* Ringer was replaced by jugular blood and this at *35* by jugular blood to which was added adrenalin to make a concentration of 1 : 95,000,000. The bloods were diluted with one volume Ringer (the adrenalin blood after adding the adrenalin). As in all the other figures, time is marked in half-minutes. (Reduced to one-half.)

The epinephrin assay of the adrenal blood (samples of the tracings are given in fig. 1) showed that the 4th specimen was much weaker than 1: 62,500,000 adrenalin[1] (observations 7 and 11). Other tracings, not reproduced, indicated that it did not differ much from 1: 125,000,000. The 2nd and 3rd specimens, corresponding to the greater flow, were weaker than the 4th, the 2nd being decidedly weaker than 1: 125,000,000 adrenalin. This was confirmed by several observations (not reproduced). In figure 1 it is shown that the 3rd specimen was distinctly weaker than 1: 95,000,000 adrenalin (observations 33 and 35).

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[1] All our results on the concentration and output of epinephrin are given in terms of the base, the amount of the base in Parke, Davis & Co.'s adrenalin hydrochloride solution being assayed by the colorimetric method of Folin, Cannon and Denis. The assay of freshly opened bottles has usually yielded from 65 to 80 per cent adrenalin base. A bottle which is used more than once is always re-assayed from time to time.

TABLE 1

NUMBER OF DOG	BODY WEIGHT	WEIGHT OF ADRENALS	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN	
			Grams	Minutes	Seconds	Grams per minute		Per animal per minute	Per kilogram per minute
	kgm.	*grams*						*mgm.*	*mgm.*
15	8.5		24	3		8.0	1: 3,500,000	0.0024	0.0002
			22	3		7.3	1: 3,000,000	0.0024	0.0002
17	6.7	0.65	20	4	45	4.2	1: 3,300,000	0.0013	0.0002
18	7.7	0.78	13	2		6.5	1: 6,000,000	0.001	0.00015
206	6.2	0.905	22.9	2		11.5	1: 7,500,000	0.0015	0.00025
207	7.3	1.2	16.1	2		8.0	1: 8,000,000	0.001	0.00014
221	11.3	1.8	17.4		30	34.8	1: 18,000,000	0.002	0.00018
245	9.5	1.55	10.0	1		10.0	1: 3,750,000	0.0027	0.00028
246	7.5	1.26	7.7		30	15.4	1: 13,000,000	0.0012	0.00016
247	7.25	1.12	4.8	1	30	3.2	1: 1,800,000	0.0018	0.00025
248	4.6	0.8	9.7	1		9.7	1: 13,000,000	0.0008	0.00017
249	6.6	0.848	9.6	1		9.6	1: 6,250,000	0.0015	0.00023
256	6.2	0.733	10.6	1	30	7.1	1: 6,500,000	0.0011	0.00018
257	6.0	0.908	8.65	1		8.65	1: 6,650,000	0.0013	0.0002
263	4.4	0.78	6.15	1		6.15	1: 3,800,000	0.0016	0.00036
280	7.0	1.8	12.5	1		12.5	1: 10,000,000	0.0013	0.00018
306	5.05	0.705	8.2	1		8.2	1: 8,000,000	0.001	0.0002
307	4.6	0.831	11.05	1	30	7.4	1: 4,800,000	0.0015	0.00033

The first three dogs in the table were anesthetized with ether alone, the rest with morphine and ether.

Taking the 4th adrenal specimen at 1: 120,000,000 we get an output of 0.0000065 mgm. per minute for the dog, or 0.0000009 mgm. per kilogram of body weight per minute. The normal average output for dogs under the conditions of our experiments, as assayed on rabbit intestine (and uterus) segments, is 0.00022 mgm. per kilogram per minute (table 1). The output had, therefore been reduced by the operation in this animal to less than $\frac{1}{200}$ of the normal.

CONDENSED PROTOCOL

Dog 242. Female. Weight 5.21 kgm.

October 28, 1918. Right adrenal excised. It weighed 0.425 gram and contained 0.47 mgm. epinephrin. Nerves of left adrenal cut (major and minor splanchnics and all branches seen entering gland; superior mesenteric ganglion and part of coeliac ganglion also excised).

November 22, 1918. Condition excellent. Weight 5.6 kgm. Under morphine and ether collected jugular blood, then made cava pocket in usual way, tying abdominal aorta but not the coeliac and mesenteric arteries.

Collected adrenal blood:

11.35 a.m. 1st specimen 0.6 gram in 30 seconds (1.2 grams per minute). 2nd specimen 6.15 grams in 5 minutes (1.2 grams per minute)

11.55 a.m. Injected intravenously 0.3 mgm. strychnine sulphate—clonic spasms

11.56 a.m. 3rd specimen, 0.75 gram in 30 seconds (1.5 grams per minute). 4th specimen, 6.05 grams in 4 minutes (1.5 grams per minute)

12.36 p.m. 5th specimen, 0.55 gram in 30 seconds (1.1 grams per minute). 6th specimen, 5.9 grams in 6 minutes (1.0 gram per minute)

Now obtained more indifferent blood from abdominal aorta.

Left adrenal weighed 0.491 gram and contained 0.58 mgm. epinephrin

The epinephrin assay of the adrenal vein blood (a few of the tracings are reproduced in fig. 2) gave no indication that any epinephrin whatever was present, although the segments used for the assay were more than usually sensitive. We have shown elsewhere (3) that strychnine causes a marked and sustained increase in the rate of liberation of epinephrin, doubtless through the nervous mechanism. Yet neither before nor after strychnine could any epinephrin be detected in the adrenal vein blood. It was found that with indifferent blood made up to a concentration of 1:165,000,000 adrenalin and then diluted with three volumes of Ringer's solution, adrenalin was easily detected. Indifferent blood made up to a concentration of 1:330,000,000 adrenalin and then diluted with its own volume of Ringer produced a definite inhibition of the intestine segment. When the weight on the lever was increased the reaction became still more delicate, so that indifferent blood made up with adrenalin to a concentration of 1:500,000,000 and then diluted with an equal volume of Ringer's solution gave a distinct effect (observation 29, fig. 2), as did a concentration of 1:650,000,000 (observation 33). Even a concentration of 1:800,000,000 could be plainly detected (observation 35, fig. 2), while the 6th adrenal blood specimen similarly diluted gave no inhibition whatever (observation 31). Numerous additional observations (not reproduced) were made, but no evidence was obtained that any of the adrenal specimens contained epinephrin.

The output could not have been as much as 0.000001 mgm. per minute for the dog, or 0.0000002 mgm. per kilogram per minute, i.e. not $\frac{1}{1000}$ of the normal output. The section of the nerves was, therefore, complete. The failure of strychnine to excite any detectable secretion confirms this conclusion.

Although in these experiments the condition of the animals at the time they were sacrificed for the determination of the residual output of epinephrin was so good that there could be no reasonable doubt that they would have survived indefinitely, the next dog was allowed to live for a longer period (eighty days). For the determination of the degree in which the epinephrin output has been suppressed by the operation this longer survival period has one disadvantage, namely, the possibility that regeneration of the secretory fibers may have occurred before the residual output of epinephrin is estimated.

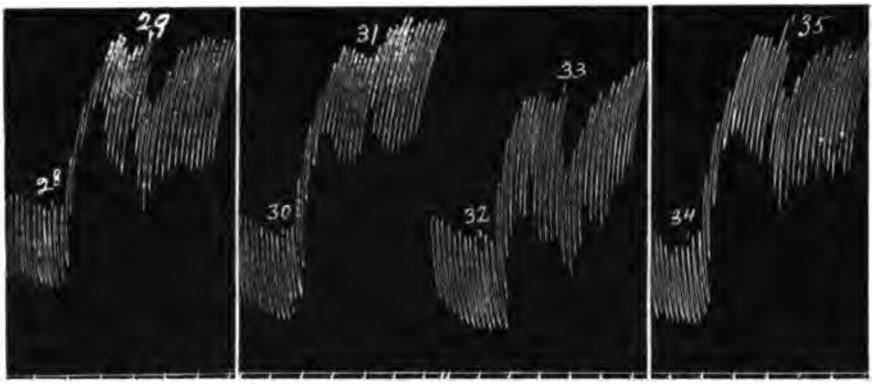

Fig. 2. Intestine tracings. Bloods from dog 242. At *28*, *30*, *32* and *34* Ringer was replaced by indifferent blood to which was added adrenalin to make a concentration of 1: 500,000,000; at *31* by the 6th adrenal specimen; at *33* by indifferent blood to which was added adrenalin to make a concentration of 1: 650,000,000 and at *35* by indifferent blood to which was added adrenalin to make a concentration of 1: 800,000,000. All the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

CONDENSED PROTOCOL

Dog 243. Male. Weight 6.5 kgm.

October 28, 1918. Right adrenal excised. It weighed 0.428 gram. Nerves of left adrenal cut as in dog 241. The animal made a rapid recovery.

January 16, 1919. Condition excellent. Weight 7.6 kgm.

Under morphine and ether obtained a sample of jugular blood. Then made cava pocket as in the other dogs, and collected adrenal blood.

12.45 p.m. 1st specimen, 2.05 grams in 30 seconds (4.1 grams per minute). 2nd specimen, 8.35 grams in 2 minutes (4.1 grams per minute)

12.50 p.m. Injected 0.5 mgm. strychnine sulphate intravenously. Tonic convulsions. Artificial respiration started. Collected adrenal blood.

12.50½ p.m. 3rd specimen, 1.15 grams in 30 seconds (2.3 grams per minute). 4th specimen, 5.5 grams in 2 minutes (2.75 grams per minute)

1.05 p.m. Convulsions ceased; spontaneous respiration going on, with hyperpnoea. Stopped artificial respiration.

1.15 p.m. Reflexes still exaggerated. Collected adrenal blood. 5th specimen, 2.5 grams in 45 seconds (3.3 grams per minute). 6th specimen, 6.9 grams in 2 minutes (3.45 grams per minute)

Now obtained blood from abdominal aorta. Left adrenal weighed 0.49 gram.

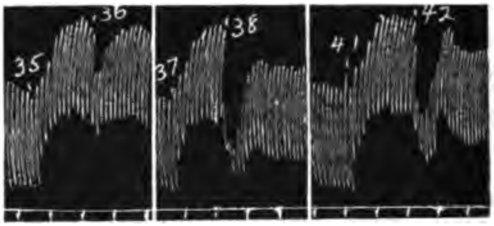

Fig. 3. Intestine tracings. Bloods from dog 243. At *35*, *37* and *41* Ringer was replaced by jugular blood and this at *36* by jugular blood to which was added adrenalin to make a concentration of 1: 10,000,000; at *38* by jugular blood to which was added adrenalin to make a concentration of 1: 8,000,000; at *42* by the 2nd adrenal specimen. All of the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

The epinephrin assay showed that the 2nd adrenal specimen had a concentration less than 1: 7,000,000 and greater than 1: 10,000,000 (fig. 3, observations 36 and 42—confirmed by several pairs of observations not reproduced). Observation 38 (fig. 3) indicates that the 2nd specimen was somewhat less than 1: 8,000,000. The 4th specimen (taken after strychnine injection) had a much greater concentration than 1: 7,000,000 and was stronger than 1: 4,000,000 (fig. 4, observations 44 to 56). Figure 5 (observations 58, 60 and 62) shows that the 4th specimen did not differ much from 1: 2,800,000. It was decidedly stronger than 1: 4,000,000 and much weaker than 1: 1,300,000 (fig. 5, observation 64). Qualitatively the 4th specimen was found to be much stronger than the 2nd (intestine observations not reproduced, confirmed by uterus observations 80 and 81, fig. 6). The assay indicates that the difference between the 4th and 2nd specimens is more than can be accounted for by the difference in the blood-flows. Taking the 2nd specimen at 1: 9,000,000, we get 0.00045 mgm. per minute for the dog, or 0.00006 mgm. per kilogram per minute, i.e., ⅓ to ¼ of the

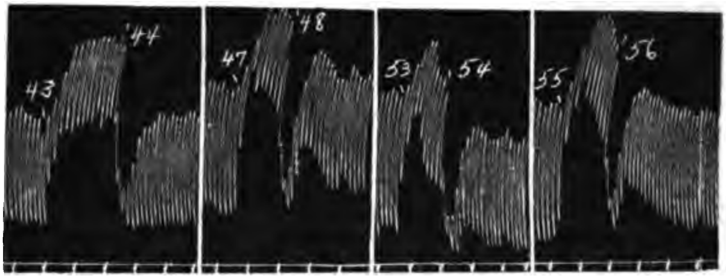

Fig. 4. Intestine tracings. Bloods from dog 243. At *43*, *47*, *53* and *55* Ringer was replaced by jugular blood, and this at *44* and *54* by the 4th adrenal specimen; at *48* by jugular blood to which was added adrenalin to make a concentration of 1: 7,000,000; at *56* by jugular blood to which was added adrenalin to make a concentration of 1: 4,000,000. All the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

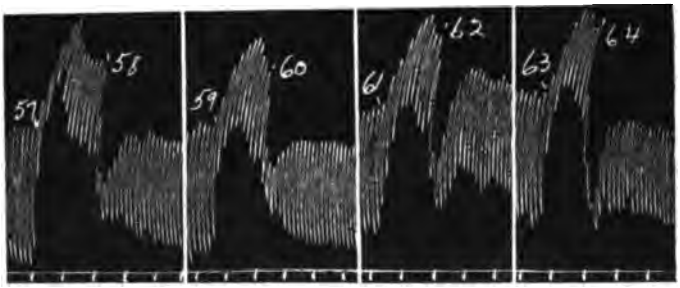

Fig. 5. Intestine tracings. Bloods from dog 243. At *57*, *59*, *61* and *63* Ringer was replaced by arterial blood and this at *58* by arterial blood to which was added adrenalin to make a concentration of 1: 2,800,000; at *60* by the 4th adrenal specimen; at *62* by arterial blood to which was added adrenalin to make a concentration of 1: 4,000,000; at *64* by arterial blood to which was added adrenalin to make a concentration of 1: 1,300,000. The bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

normal output for both adrenals. This would correspond to ½ to ⅔ the normal output for the denervated adrenal. As we have never found so great an output after this operation when the assay was made two or three weeks after the operation, the suggestion is that some regeneration must have occurred. This conclusion is strengthened by the fact that strychnine seems to have increased the output. Thus, if the 4th specimen is taken at 1:2,800,000, the output per minute after strychnine would be 0.001 mgm. for the dog or 0.00013 mgm. per kilogram per minute, about double the initial output. The increase is not very great compared with that usually caused by strychnine in a normal animal, but this may be because regeneration was incomplete at the time the animal was sacrificed. In any case we should not venture to draw from this experiment the definite conclusion that regeneration of these fibers had occurred to a substantial extent in the eighty days since the operation. It is always possible in the present state of our knowledge of these secretory paths that owing to some anomaly a large portion of the fibers was spared by our operation. Our only ground for considering this to be improbable is that we have not encountered the condition when the interval of survival was only a few weeks.

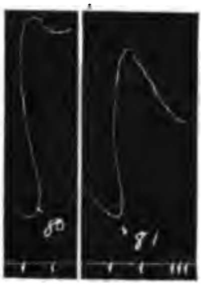

Fig. 6. Uterus tracings. Bloods from dog 243. At *80* Ringer was replaced by the 4th adrenal specimen; at *81* by the 2nd adrenal specimen. The bloods were diluted with ten volumes Ringer. (Reduced to one-half.)

The normal output of epinephrin was estimated in two monkeys.

CONDENSED PROTOCOL

Macacus cynomolgos.[2] *Female. Weight 2.51 kgm.*

Under morphine and urethane a sample of blood was drawn from the internal jugular vein. Then the cava pocket was made as in the other experiments, the abdominal aorta being tied, but not the coeliac or mesenteric arteries. Adrenal blood was collected:

1st specimen, 3.4 grams in 1 minute (3.4 grams per minute)
2nd specimen, 7.0 grams in 3 minutes (2.33 grams per minute)
3rd specimen, 4.95 grams in 3 minutes (1.65 grams per minute)

More jugular and some arterial blood was now obtained. The right adrenal weighed 0.286 gram, the left 0.283 gram.

It was found at autopsy that a vein coming from the upper pole of the right adrenal passed up through the liver before entering the cava. The blood from this vein which, from its size, probably carried about one-third of the blood from that adrenal, would not be collected in clipping off the cava pocket. This vein was seen in the other monkeys operated on and must always be taken account of. The main adrenal vein from the right gland entered the cava at about the level usual in the dog and the blood from it could be collected.

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[2] In a previous paper this animal, an immature specimen showing complete permanent set of teeth with exception of the third molars above and below, was incorrectly referred to as a baboon.

It was shown on rabbit segments that the 3rd specimen was stronger than the 2nd, corresponding to the smaller flow; that the 2nd and 3rd specimens were stronger than 1: 7,000,000 and weaker than 1: 2,750,000 adrenalin; that the 3rd specimen was somewhat stronger than 1: 4,800,000 and somewhat weaker than 1: 4,000,000 while the 2nd specimen was somewhat weaker than 1: 5,500,000.

Taking the 2nd specimen at 1: 6,000,000 we get an output of 0.0004 mgm. per minute for the animal, or correcting for the loss of a portion of the blood from the right adrenal, say 0.0005 mgm., i.e., 0.0002 mgm. per kilogram of body weight per minute. Taking the 3rd specimen at 1: 4,300,000, we get the same output.

In another monkey (Macacus rhesus) weighing 8.4 kilograms the output with an adrenal blood flow of 7.5 grams per minute was 0.0013 per minute for the animal, or 0.00015 mgm. per kilogram per minute. The right adrenal weighed 0.465 gram. The left adrenal weighed 0.48 gram.

In two monkeys (Macacus rhesus) the adrenal operation (excision of the right gland, denervation of the left) was performed in the usual manner. One of the animals was sacrificed after twenty-two days for estimation of any residual epinephrin output. It was at that time in excellent condition and, so far as could be made out, unaffected in any way by the loss of the epinephrin secretion. The other monkey[a] was allowed to survive and is still alive and well five months after the operation. His weight is precisely the same as at the time of the operation. The condensed protocol of the monkey which was sacrificed follows:

CONDENSED PROTOCOL

Macacus rhesus. Male.

October 29, 1918. Right adrenal excised. It weighed 0.325 gram and contained 0.52 mgm. epinephrin. The left adrenal was denervated. Recovery was rapid, and the animal was active the next day.

November 20, 1918. Condition excellent. Weight 4.1 kgm.

Under urethane obtained indifferent (internal jugular) blood. Then made cava pocket as in the other animals. Collected adrenal blood.

1st specimen, 2.8 grams in 1 minute (2.8 grams per minute)
2nd specimen, 5.45 grams in 2 minutes (2.72 grams per minute)
3rd specimen, 7.05 grams in 3 minutes (2.35 grams per minute)

4th specimen, 5.75 grams in 3 minutes (1.91 grams per minute)

Now obtained more venous blood through cannula pushed into right heart through inferior cava and also some arterial blood. Left adrenal weighed 0.442 gram and contained 0.66 mgm. epinephrin.

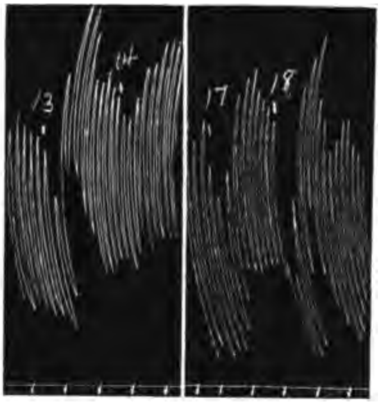

Fig. 7. Intestine tracings. Bloods from monkey, whose right adrenal was excised and left adrenal denervated 22 days prior to experiment. At *13* and *17* Ringer was replaced by venous blood and this at *14* by the 4th adrenal specimen; at *18* by venous blood to which was added adrenalin to make a concentration of 1: 130,000,000. The bloods were diluted with one volume Ringer (the adrenalin blood after adding the adrenalin). (Reduced to one-half.)

With the rabbit intestine and uterus segments none of the adrenal specimens gave any reaction from which the presence of epinephrin could be deduced. Samples of the tracings are reproduced in figures 7 to 9. In figure 7 it is shown that the 4th specimen could not have contained epinephrin even in a concentration of 1: 130,000,000. Even with the undiluted blood the 3rd specimen produced no definite inhibition of the intestine (fig. 8, observation 22). Another test with the 3rd specimen immediately after observation 30 gave an absolutely negative result. Yet the segment gave a distinct reaction with 1:330,000,000 adrenalin in indifferent blood (observation 30), a stronger reaction with 1: 160,000,000 and a marked effect with 1:80,000,000.

It was confirmed on the uterus that the adrenal specimens caused effects which did not differ sufficiently from those produced by indifferent blood to permit the conclusion that they contained epinephrin. Thus, the increase of tone produced by the 4th specimen was little greater than that produced by indifferent (arterial) blood (fig. 9, observations 33 and 34). The effect caused by the 3rd specimen was no greater than that caused by indifferent (venous) blood, although adrenalin in a concentration of 1: 80,000,000 in the indifferent blood gave a good rise of tone (fig. 9, observations 43 to 45).

Since our last paper (1) on this subject was published we have had the opportunity, in connection with other researches, of estimating the residual output of epinephrin after excision of one adrenal and denervation of the other in a considerable number of cats, which were allowed to survive long enough to recover completely from the effects of the operation. The results are combined in table 2, which supplements the similar data already published in tabular form in a previous paper (4).

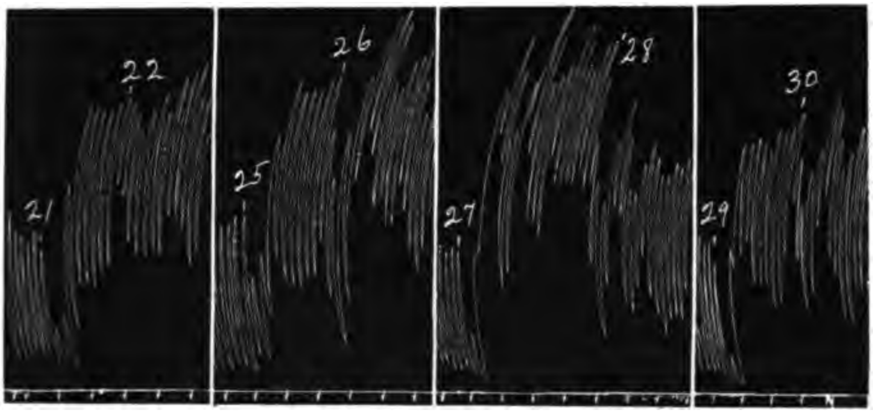

Fig. 8. Intestine tracings. Bloods from same monkey as in figure 7. At *21*, *25*, *27* and *29* Ringer was replaced by arterial blood and this at *22* by the 3rd adrenal specimen; at *26* by arterial blood to which was added adrenalin to make a concentration of 1: 160,000,000; at *28* by arterial blood with adrenalin to make a concentration of 1: 80,000,000; at *30* by arterial blood with adrenalin added to make a concentration of 1: 330,000,000. The bloods were not diluted before application to the segment. (Reduced to one-half.)

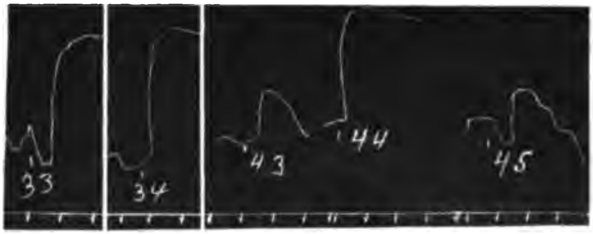

Fig. 9. Uterus tracings. Bloods from same monkey as in figures 7 and 8. At *33* Ringer was replaced by arterial blood; at *34* by the 4th adrenal specimen. The bloods were diluted with five volumes Ringer. With another uterus segment, at *43* Ringer was replaced by the 3rd adrenal specimen; at *44* by venous blood with adrenalin added to make a concentration of 1:80,000,000, and at *45* by venous blood. The bloods were diluted with one volume Ringer (the adrenalin blood after adding the adrenalin). (Reduced to one-half.)

Five cats, not included in the table, were sacrificed in experiments which did not permit an assay of the adrenal blood. They had survived for a period of from ten to sixty-two days in good health.

Since it is important for estimating the degree in which the epinephrin output has been affected by denervation or by any other procedure, to establish on the basis of as large a number of observations as possible the normal average output and the normal range, additional data on cats are collected in table 3. All the assays given in tables 1 to 3 were made on rabbit intestine (and uterus) segments.

TABLE 2

NUMBER OF CAT	BODY WEIGHT	EPINEPHRIN OUTPUT		FRACTION OF NORMAL LIBERATION	DAYS AFTER OPERATION
		Per minute	Per kilogram per minute		
	kgm.	*mgm.*	*mgm.*		
201	2.575	0.000012	0.0000045	$\frac{1}{50}$	18
202	2.545	0.000007	0.000003	$\frac{1}{100}$*	8
215	2.38	0.00007	0.00003	$\frac{1}{10}$	59
219	2.54	0.000046	0.000018	$\frac{1}{12}$	27
231	3.08	0 00003	0.00001	$\frac{1}{25}$*	33
233	2.62	0.000033	0.000013	$\frac{1}{20}$	36
235	2.25	0.000002	0.0000009	$\frac{1}{250}$*	40
277	1.75	0.000055	0.00003	$\frac{1}{8}$†	21
304	2.15	0.000033	0.000016	$\frac{1}{15}$	46

* In these cats there was no evidence that any epinephrin was being liberated. The amount given in the table is such as could have been detected by the test objects.

† Strychnine was administered before collection of the adrenal blood; this may have increased the output, so that the amount indicated by the assay is probably higher than the actual residual liberation.

In cat 215, assay by eye reactions gave an output of 0.00014 mgm. per minute for the animal, or 0.00006 mgm. per kgm. per minute, i.e., about $\frac{1}{4}$ of the normal average. In cat 233, assay by eye reactions gave an output of 0.00006 mgm. per minute for the animal, or 0.000023 mgm. per kilogram per minute, i e., $\frac{1}{10}$ of the normal average.

The average output of epinephrin per kgm. of body weight per minute for the urethanized cats is 0.00025 mgm., the same as that given in the previous paper (1). The average for the 16 etherized cats is 0.0002 mgm. The average for the 45 cats included in table 2 of the previous paper and table 3 of this paper is 0.00023 mgm. per kgm. per minute.

TABLE 3

NUMBER OF CAT	BODY WEIGHT	WEIGHT OF ADRENALS	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN	
			Grams	Minutes	Seconds	Grams per minute		Per animal per minute	Per kilogram per minute
	kgm.	*grams*						*mgm.*	*mgm.*
204	3.25	0.60	6.4		30	12.8	1: 14,000,000	0.0009	0.00027
205	2.7	0.68	7.3	3		2.4	1: 4,000,000	0.0006	0.0002
210	2.9	0.583	3.7	7		0.5	1: 800,000	0.0006	0.0002
211	2.8	0.58	3.5	4		0.9	1: 2,000,000	0.0005	0.0002
212	2.2	0.496	4.3	3		1.43	1: 3,200,000	0.00045	0.0002
213	2.67	0.468	9.8	3		3.3	1: 3,500,000	0.001	0.00037
214	2.35	0.348	7.0	3		2.3	1: 2,000,000	0.0011	0.00045
222	2.17	0.298	7.0	3		2.3	1: 5,500,000	0.0004	0.0002
223	3.47	0.462	6.1	2	30	2.45	1: 2,500,000	0.001	0.0003
225	2.05	0.292	3.7	3		1.2	1: 2,400,000	0.0005	0.00025
228	2.5	0.492	5.2	1	30	3.5	1: 5,800,000	0.0006	0.00024
229	2.07	0.387	4.7	3		1.6	1: 2,200,000	0.0007	0.00035
230	2.08	0.412	4.75	3		1.6	1: 2,500,000	0.0006	0.0003
238	1.64	0.288	5.3	3		1.7	1: 5,000,000	0.00034	0.0002
239	2.41	0.35	4.6	2		2.3	1: 5,000,000	0.00046	0.0002
258	2.76	0.323	6.05	3		2.0	1: 4,800,000	0.00042	0.00015
259	4.1	0.73	9.05	2		4.5	1: 4,500,000	0.001	0.00025
281	4.37	0.553	7.8	2		3.9	1: 4,500,000	0.00087	0.0002
282	3.1	0.51	5.7	2		2.9	1: 3,000,000	0.0009	0.0003
283	3.38	0.33	5.55	2		2.8	1: 7,000,000	0.0004	0.00012
284	2.85	0.502	4.0	2		2.0	1: 7,000,000	0.0003	0.0001
285	4.4	0.553	9.2	2		4.6	1: 6,500,000	0.0007	0.00016
286	4.0	0.372	7.1	1	30	4.7	1: 6,500,000	0.00072	0.00018
287	3.71	0.376	4.8	3		1.6	1: 6,500,000	0.00035	0.0001
288	2.175	0.37	4.2	2		2.1	1: 3,800,000	0.00055	0.00025
289	2.83	0.342	6.0	3		2.0	1: 1,300,000	0.0015	0.0005
290	2.265	0.296	3.3	3		1.1	1: 2,000,000	0.00055	0.00025
292	2.07	0.278	2.35	5		0.5	1: 1,300,000	0.0004	0.0002
293	3.865	0.377	8.8	1	30	5.87	1: 9,000,000	0.00065	0.00017
294	2.73	0.316	6.85	1	30	4.8	1: 6,600,000	0.0007	0.00025
295	2.9	0.372	6.45	2		3.2	1: 8,800,000	0.0005	0.00017
296	2.0	0.215	3.7	4		0.92	1: 2,300,000	0.0004	0.0002
298	3.35	0.418	5.35	3		1.8	1: 3,000,000	0.0006	0.00018
303	4.82	0.66	9.05	1	30	6.0	1: 6,000,000	0.001	0.0002
305	3.41	0.312	6.8	2		3.4	1: 6,000,000	0.00057	0.00017

The first 19 cats in the table (down to and including cat 282) were anesthetized with urethane, the rest with ether.

CONCLUSION

In dogs and monkeys, as previously shown for cats and rabbits, the liberation of epinephrin from the adrenals is not indispensable for life and health.

BIBLIOGRAPHY

(1) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1917, xi, 1.
(2) Stewart and Rogoff: This Journal, 1918, xlvi, 90.
(3) Stewart and Rogoff: This Journal, 1919, xlviii, 22; Journ. Pharm. Exper. Therap., 1919, xiii.
(4) Stewart and Rogoff: This Journal, 1917, xliv, 543.

THE GASTRIC RESPONSE TO FOODS

II. A FRACTIONAL STUDY OF THE COAGULATION OF MILK IN THE HUMAN STOMACH[1]

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In the course of our experiments upon the gastric response to foods, we were particularly fortunate in securing the coöperation of a normal man, thirty-one years of age, who had the unique ability to deliver samples of stomach contents at will. Not only could this man completely empty his stomach at any desired stage in the process of digestion but he could also give us small samples at short intervals throughout the entire gastric cycle. In other words, we could make a fractional examination of his stomach contents without recourse to a stomach tube. Inasmuch as this regurgitator was to be available for only a short time, we decided to study milk coagulation at the outset of our observations. These studies told us much of interest. For example, we learned that raw whole cows' milk forms a large hard curd whereas boiled milk curds in a much finer and softer form; that the presence of much cream (milk fat) in the milk insures the formation of particularly soft curds which are slow to leave the stomach; that skimmed milk yields a particularly hard curd due to the absence of fat; that pasteurized milk shows smaller curds than the raw whole milk but larger than the boiled whole milk; that cold milk coagulated more slowly than warm milk, and that milk drunk very slowly, or "sipped," curds in larger curds and leaves the stomach more slowly than similar milk drunk very rapidly.

The only other experiments in which a regurgitator was used in the study of milk curding, so far as we are aware, are those of Brennemann (1). However in the case of Brennemann's work no attempt was made to follow the milk through the various stages of its coagula-

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tion, the experimental procedure simply embracing the *complete* emptying of the stomach at a certain time (usually thirty minutes) after drinking the milk. He did, however, get a line upon the curding sequence in certain cases by emptying the stomach at different intervals upon successive days. In spite of the variation in our plan of experimentation we have nevertheless been able, wherever our investigation overlapped that of Brennemann, to verify him in remarkable detail.

EXPERIMENTAL

Studies of two kinds were made—one in which small samples of stomach contents were regurgitated at intervals throughout the curding period of the milk, the other in which the stomach was completely emptied a half-hour after a pint of milk had been ingested. Unless otherwise stated the experiments were made before breakfast on an empty stomach. Typical protocols follow:

Experiment 1. Raw whole milk.

Five hundred cubic centimeters of milk with a temperature of 37°C. were drunk in thirty seconds. Eleven samples were regurgitated at intervals for one hour, the first regurgitation taking place one-half minute after the milk was taken.

Remarks. Small curds were regurgitated one-half minute after the milk was drunk. These curds were white in color and those returned thirty to forty minutes after drinking the milk were firm and rubber-like in texture and as large as an average size peanut. At the end of the hour tough curds as large as a man's thumb were in evidence. (See exper. 14. For photographs of curds, see fig. 1a).

Experiment 2. Boiled whole milk.

Five hundred cubic centimeters of milk were boiled for five minutes, then cooled to 37°C. and drunk in ten seconds. Ten samples were regurgitated at intervals for one hour, the first one at the one-minute period.

Remarks. In the case of boiled milk the curds were much smaller and softer than were the curds from raw whole milk. They were also not white as were the raw milk curds but possessed a yellowish tint. The maximum size of the boiled milk curds was about that of a small pea. (See fig. 2).

Experiment 3. Raw skimmed milk.

Five hundred cubic centimeters of milk, temperature 37°C., were taken in ten seconds. Ten samples were regurgitated, the first being returned thirty seconds after the milk was drunk.

Remarks. Large curds formed more quickly from skimmed milk than from whole milk. In one-half minute after the milk was drunk, small curds began to form. Within five minutes white curds as large as a man's thumb were regurgitated. These curds were firmer and more rubber-like than those from any other kind of milk tested. (For pictures see fig. 3).

Experiment 4. Influence of water.

In this test 500 cc. distilled water (temp. 25°C.) were drunk in five minutes, and after an interval of three minutes 500 cc. of raw whole milk (temp. 37°C.) were also drunk in five minutes. Eleven samples were regurgitated during the next hour, the first one being returned thirty seconds after the drinking of the milk.

Remarks. The diluted milk coagulated about the same as the undiluted milk except that the maximum curd formation was hardly as large (See exper. 1). (For photographs of curds, see fig. 4).

Experiment 5. Influence of sodium bicarbonate.

Five hundred cubic centimeters of raw whole milk (temp. 37°C.) to which had been added 2.5 grams of sodium bicarbonate were drunk in thirty seconds. Eleven samples were regurgitated, the first one at the half-minute interval.

Remarks. In spite of the fact that the stomach contents were alkaline up to one-half hour after the milk was drunk, definite curds began to form at the five-minute interval. The curds were smaller and softer than the curds of raw whole milk (exper. 1) but not so soft and flaky as were the curds from the boiled whole milk (exper. 2). (For pictures of curds see fig. 5).

Experiment 6. Cream (20 per cent fat).

Five hundred cubic centimeters of cream containing 20 per cent milk fat (temp. 37.5°C.) were drunk in ten seconds. Nine samples were regurgitated at intervals for two hours and a half, the first sample being collected one-half minute after drinking the cream.

Remarks. Small soft curds reaching their maximum size at about twenty minutes (see fig. 6). Stomach emptied slowly due to presence of fat. (See exper. 11).

Experiment 7. Mixture of boiled and raw milk.

Four hundred cubic centimeters of raw whole milk were boiled for five minutes, then cooled to 37°C. To this was added 100 cc. of the original raw milk and the mixture drunk in ten seconds. Eleven samples were regurgitated in an hour, the first one at the one-half minute interval.

Remarks. Notwithstanding the fact that 80 per cent of the milk was *boiled*, the mixture curded more after the manner of *raw* milk. Fairly large curds were formed in twenty minutes. At no time were the soft, fluffy curds of boiled milk in evidence. (See fig. 7).

Experiment 8. Influence of water.

In this test 500 cc. distilled water (temp. 37°C.) were drunk in fifteen seconds, followed immediately by 500 cc. raw whole milk of same temperature, also drunk in fifteen seconds. Twelve samples were collected during the following hour, the first one being regurgitated at the one-half minute interval.

Remarks. There was less curd formation in this experiment than in experiment 4 and the stomach apparently emptied somewhat more slowly.

Experiment 9. Boiled skimmed milk.

Five hundred cubic centimeters of skimmed milk (sp. gr. 1034 at 10°C.) were boiled for five minutes, then cooled to 37°C. and drunk in ten seconds. Eleven samples were subsequently regurgitated within one hour, the first sample being returned in one-half minute from the time the milk was ingested.

Remarks. Curds began to form in one-half minute, but these curds were smaller than in the case of raw skimmed milk (see exper. 3). In fact they never became larger than a small kidney bean. The curds were, however, larger than those regurgitated from the boiled whole milk (see exper. 2). (For photographs see fig. 8).

Experiment 10. Pasteurized milk.

Five hundred cubic centimeters of pasteurized whole milk (sp. gr. 1029 and temp. 20°C.) were drunk in ten seconds. Eleven samples were collected in one hour, the first specimen being regurgitated one-half minute after the drinking of the milk.

Remarks. Small curds, formed at the two and one-half minute period. Maximum curd formation at forty minutes. The curds were intermediate in character between those of raw and boiled milk, but more nearly approached the raw milk type. (See curds in fig. 9).

Experiment 11. Cream (40 per cent fat).

Five hundred cubic centimeters of pasteurized cream (40 per cent fat) having a temperature of 37.5°C. were drunk in ten seconds. Fourteen samples were collected during an interval of two and one-half hours, the first sample being regurgitated at the one-half minute interval.

Remarks. No curd formation until one-half hour after the cream was drunk. Even then the curds were very small (pinhead) and did not increase in size thereafter. The stomach emptied very slowly and two and one-half hours after 500 cc. of the cream were drunk, 475 cc. of material were regurgitated (see exper. 6). (For photographs see fig. 10).

Experiment 12. Milk drunk slowly.

Five hundred cubic centimeters of raw whole milk at 20°C. were drunk in *ten minutes* and the stomach completely emptied by regurgitation one-half hour later. (For photograph of the curds see figs. 11 and 12). Experiment 13 followed immediately.

Remarks. There was more curd regurgitated one-half hour after 500 cc. of milk were drunk slowly than when a similar volume of milk was taken rapidly. The individual curds were also larger. (See expers. 13, 15 and 16).

Experiment 13. Milk drunk rapidly.

Five hundred cubic centimeters of raw whole milk at 20°C. were drunk in *fifteen seconds* and the stomach completely emptied by regurgitation one-half hour later. (For photograph of the curds see figs. 11 and 12).

Remarks. The total mass of curd regurgitated one-half hour after drinking 500 cc. of milk rapidly was less than after a similar volume of milk had been drunk slowly. The curds were also smaller. (See expers. 12, 15 and 16).

Experiment 14. Raw whole milk.

Five hundred cubic centimeters of raw whole milk, 20°C., drunk in seventeen seconds. Eight samples were regurgitated in forty minutes, the first one being collected one minute after drinking the milk.

Remarks. Curds began to form in one minute and in twenty-five minutes a curd weighing 15 grams was regurgitated. This curd was very tough and "rubbery." (See exper. 1). (For pictures of curds see fig. 1).

Experiment 15. Milk drunk rapidly.

Five hundred cubic centimeters raw whole milk, 20°C., drunk in fifteen seconds. One-half hour later the stomach was completely emptied by regurgitation. Experiment 16 followed immediately.

Remarks. This experiment is a duplicate of experiment 13 except that it preceded the test on the slow drinking of milk (see exper. 16) instead of following that test as in the case of experiment 13. At the end of the experiment 160 cc.

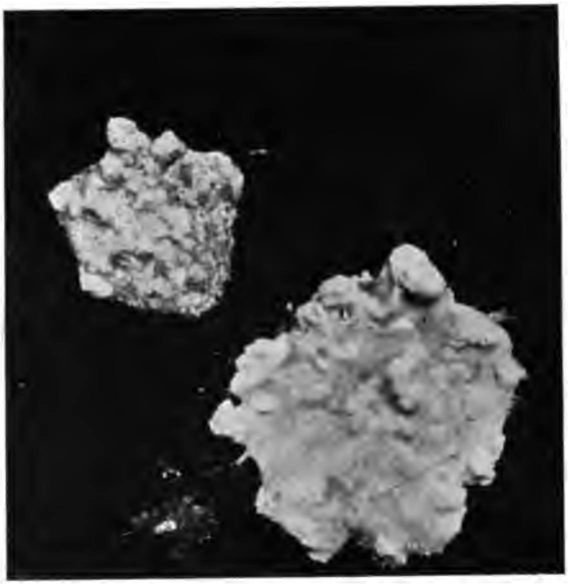

Fig. 11. Large curd mass = 500 cc. raw whole milk at 20°C. drunk in *10 minutes*. Small curd mass = 500 cc. raw whole milk at 20°C. drunk in *15 seconds*. (0.5 natural size.)

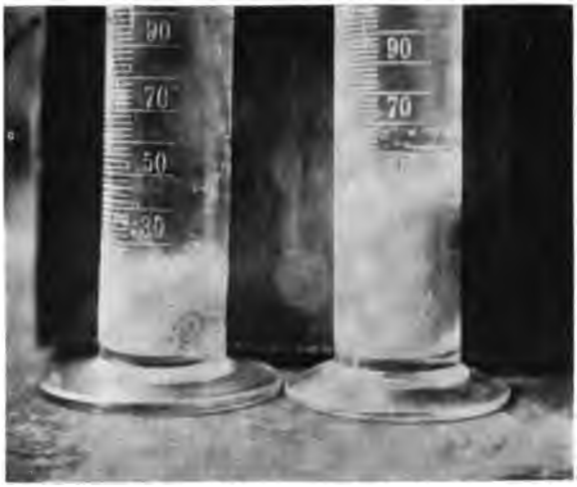

Fig. 12. Large curd mass = 500 cc. raw whole milk at 20°C. drunk in *10 minutes*. Small curd mass = 500 cc. raw whole milk at 20°C. drunk in *15 seconds*.

of water were drunk and by regurgitation 160 cc. of fluid with a few suspended curds were obtained.

Experiment 16. Milk drunk slowly.

Five hundred cubic centimeters of raw whole milk, 20°C., were drunk in nine and one-half minutes. One-half hour later the stomach was emptied completely by regurgitation.

Remarks. This experiment is a duplicate of no. 12 except that it followed the rapid drinking of milk instead of preceding it as in the case of experiment 12. The results were similar in the two cases.

DISCUSSION AND SUMMARY

Rapid drinking vs. slow drinking of milk. Milk drunk rapidly left the stomach sooner and produced a smaller curd mass than milk drunk slowly or "sipped." For photographs of curds which were regurgitated one-half hour after drinking 500 cc. of raw whole milk, see figures 11 and 12.

Raw vs. boiled milk. Raw whole milk in the human stomach formed firm white curds which had "rubber-like" characteristics. The maximum curd formation took place at about one hour after the milk had entered the stomach. These large curds were apparently formed through the coalescing of numerous smaller curds. At this period curds as large as a man's thumb and weighing at least 15 grams may be formed.

Milk which had been boiled five minutes curded in an entirely different manner. In this case the curds were small, soft, flaky and of a yellow color. The largest curds were about the size of a small pea. These curds left the stomach sooner and were much more digestible than the tough curds resulting from the ingestion of the raw milk. Dietetically, therefore, boiled milk is to be preferred to the raw product. The boiling of milk for five minutes, as in our tests, did not materially alter its nutritive value or growth-promoting properties (2) but may have lowered the antiscorbutic value (3 and 4).

Skimmed vs. whole milk. Particularly tough, hard curds of large size were formed in the human stomach after the entrance of raw skimmed milk. These curds formed very quickly (one-half minute) and inside of five minutes they coalesced to form curds as large as a man's thumb. The skimmed milk curds, because of their firm, unyielding character, were more difficult to regurgitate than were the whole milk curds. The regurgitator also complained of a "heavy" feeling in his stomach soon after drinking the skimmed milk.

When the skimmed milk was boiled, the resulting curds were not as large and tough as were those of the raw skimmed milk nor as small and soft as were the curds of boiled whole milk. The largest curd (one hour) was about the size of a small kidney bean. Inasmuch as the boiled skimmed milk contained only about 0.5 per cent fat (sp. gr. 1034) whereas the boiled whole milk contained about 4 per cent fat it is apparent that the fat content is an important factor in determining the character of the curd in boiled milk. The curds of the boiled whole milk were digested more rapidly than were those of the boiled skimmed milk.

Influence of sodium bicarbonate on curd formation. The addition of two and one-half grams of sodium bicarbonate to 500 cc. of raw whole milk caused the formation of curds which were smaller and softer than those produced in similar milk in the absence of bicarbonate. There was a definite curd formation at five minutes although the stomach contents remained alkaline for thirty minutes. That the bicarbonate treatment was not as effective as was boiling in producing small soft curds was shown by the fact that the curds of the boiled whole milk were smaller and softer than were the curds in the milk after bicarbonate had been added. The boiled milk also left the stomach sooner than the bicarbonate milk.

Influence of milk fat on curd formation. As a general rule the character of the milk curds was influenced in a very direct way by the fat concentration, i.e., the more fat the less curd. Not only was there less total curd formation but the curds which were formed were smaller and softer the higher the fat concentration. In fact, when 40 per cent cream was drunk no curds were formed during the first half-hour and even then the largest curds were only about the size of the head of a pin and they did not coalesce to form larger curds as was the case in milk of low fat content.

The milks of high fat concentration were also very slow to leave the stomach. In fact two and one-half hours after 500 cc. of 40 per cent cream were ingested, at least 475 cc. of material were regurgitated from the stomach. These tests were rather distasteful to the regurgitator since the rich creams gave him a very uncomfortable, heavy feeling which bordered on nausea. There was a tendency for skimmed milk (0.5 per cent fat) to leave the stomach somewhat more slowly than the raw whole milk (4 per cent fat). We attribute this to the fact that the curds of the skimmed milk were especially tough and were slow to disintegrate under the influence of the gastric juice.

Influence of pasteurization on curd formation. Pasteurizing of milk is not nearly as effective as boiling in altering the type of curd formation. The curds from pasteurized milk were larger than those from boiled milk, but not so large as those from the raw whole milk. In short, the curds of the pasteurized milk were intermediate in character between the curds of raw and boiled milk, but had a tendency to resemble more closely the curds of raw milk. There was, however, less tendency for the pasteurized curds to coalesce with the formation of a large curd aggregate.

Influence of dilution on curd formation. The drinking of water before the milk was ingested had no material influence upon the curding tendencies of the milk, with the exception that the curds were somewhat softer and the maximum sized curds were smaller than when no water was ingested. It seems probable that the extra volume of fluid in the stomach prevented the smaller curds from coalescing with the formation of large masses.

Influence of temperature on curd formation. Milk curded very quickly in the stomach irrespective of the temperature. There was a slight slowing of the coagulation rate in the case of milks which were considerably below body temperature but the delay was not pronounced. The milks at 20°C. began to curd between one and two and one-half minutes whereas milks at 37°C. showed signs of curding at the one-half minute interval.

The curding of mixtures of boiled and raw milk. When a mixture composed of 80 per cent boiled whole milk and 20 per cent raw whole milk was drunk the resultant curds were very similar in character to those obtained from the ingestion of straight raw whole milk. Large curds were regurgitated at the twenty minute period, and the soft, yellow, fluffy curds typical of boiled milk were not in evidence.

BIBLIOGRAPHY

(1) BRENNEMANN: Journ. Amer. Med. Assoc., 1913, lx, 575. Arch. Pediatrics, 1917, xxxiv, 81.
(2) OSBORNE AND MENDEL: Journ. Biol. Chem., 1919, xxxvii, 187.
(3) HESS: Amer. Journ. Diseases Children, 1916, xii, 152; 1917, xiv, 337.
(4) CHICK, HUME AND SKELTON: Biochem. Journ., 1918, xii, 131.

STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

I. THE COLON. A CONTRIBUTION TO THE ONE-SIDED PERMEABILITY OF THE INTESTINAL WALL TO CHLORIDES[1]

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The relative part played by the physical factors of diffusion, osmosis, filtration, imbibition, etc., and the so-called "physiological activity" of the living cells, in intestinal absorption, has received much attention in the past. It is still far from a settled problem.

The apparent property of the intestine of passing dissolved substances in but one direction has been especially difficult of interpretation, if one is to assume that physical factors are chiefly concerned in intestinal absorption. This seeming power of the intestine to direct the stream of dissolved substances through the intestinal wall in but one direction, has been a subject of much investigation.

Cohnheim (1), in experiments upon dogs with a Vella fistula of the small intestine, finds that sodium chloride passes into dextrose solutions in the isolated loop only in small amount, whether the dextrose solution is isontonic, hypertonic or hypotonic. The author attributes this small amount of sodium chloride to the secretion of the intestine. It is further shown that after a sufficient interval following death, or upon injury to the cells with sodium fluoride, arsenic, etc., the normal mechanism of one-sided permeability may be disturbed since, under these conditions, the sodium chloride content of the intestinal fluid increases above normal. This, he states, proves that the permeability, in but one direction, is a function of the living intestinal epithelium. In the peritoneal and pleural cavities the author finds that sodium chloride diffuses into sugar solutions in large amount.

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[1] A preliminary report was made before the American Society of Biological Chemists, December, 1916; Journ. Biol. Chem., 1917, xxix, p. xii.

Waymouth-Reid (2) amplifies and confirms the work of Cohnheim on the one-sided permeability of the intestine. In experiments, using the mid-region of the ileum of dogs, the conclusion is reached that, except when the intestinal epithelium is injured, the small amount of sodium chloride entering the intestinal fluid originates in the succus entericus. Waymouth-Reid (3) believes that distilled water may injure the epithelium of the small intestine, but states that the lower regions are more resistant to this action than the upper. This one-sided permeability is accounted for by a "mechanism" which tends to drive the blood salts back as fast as they tend to diffuse into a bland solution lying in the gut. This same mechanism, the author states, forwards into the blood any salt entering the cells from the gut side. When sodium chloride is infused intravenously into dogs, Waymouth-Reid (3) finds that although the blood chlorides are increased, no increase in absorption of water is observed from glucose solutions in the ileum. Occasionally a decreased glucose absorption is found. Hence it is concluded that the uptake of water is not a simple function of the osmotic pressure of the sodium chloride of the blood, to which the normal epithelium is practically impermeable from the blood side to the gut side of the membrane, and since sodium chloride is the chief blood salt, there is probably no relationship to any other blood constituent. With Cohnheim the author believes that, in regard to water absorption, the osmotic factor is in relation to the action of the cells rather than to the blood.

Heidenhain (4) points out that from the ileum of dogs, solutions of sodium chloride of lower concentration than the blood (0.3 to 0.5 per cent) are absorbed, whereas if we were dealing with a purely physical phenomenon, sodium chloride should diffuse into the intestine. The conclusion is therefore drawn that while water absorption from these solutions is, in part at least, due to osmotic factors, the salt absorption is due to a "physiological activity" of the intestinal wall.

Hamburger (5), working with homogeneous membranes, the intestine of dead animals and with isolated tissues, believes to have demonstrated that the one-way permeability, together with other factors of absorption peculiar to the intestine, may be imitated in the artificial membranes and may proceed in an animal dead from one to twenty-four hours. He therefore concludes that the theory of a "physiological activity" is not justified. He would ascribe this activity to certain physical forces; molecular imbibition, intra-intestinal pressure and a suction induced by the blood stream. Therefore, the living cell is not involved.

Diena (6), using the permanently isolated colon of dogs, finds that there is a passage of substances from the interior of the organism into the intestine and vice versa. Diena reports that sodium chloride passes into strongly hypotonic solutions of this salt in the colon. This investigator further states that the quantity of such substances passing into the intestine is not related to the osmotic pressure of the liquid introduced.

The investigation to be presented was planned to determine whether the colon is impermeable to the chief blood salt, sodium chloride, in the direction of the blood into the intestinal lumen.

EXPERIMENTAL METHODS

The colon, in situ, of dogs was selected for these experiments. By the use of the colon there is assured a uniform region of the intestine. With the experimental procedure used, manipulation of the intestine is avoided thus making it possible to use the same loop repeatedly in each experiment. Furthermore, it was our hypothesis that the relatively slower absorption from the colon, than from the small intestine, might serve to allow us to study more accurately the progressive changes occurring in solutions introduced therein. E. Waymouth-Reid (7) states that absorption in the colon for all constituents of serum, for peptone and glucose, is far less per unit measured surface than in the middle region of the ileum. We are also not unaware of the possibility that qualitative as well as quantitative differences may be found, from the very nature of the structural differences of the colon and regions of the small intestine.

In a few of the experiments to be presented, decerebrate animals were employed. In others "pantopon" served as the anesthetic. Unless otherwise stated, however, paraldehyde was administered. The operation was performed under a light ether anesthesia in the two latter cases. Usually, one administration of the paraldehyde was sufficient for each animal. The dogs were kept warm throughout the period of experimentation.. The animals were prepared as follows: A mid-line incision is made in the abdomen large enough to admit two fingers. The cecum is then brought into view and a strong ligature placed around the ileum at its junction with the cecum, care being exercised not to involve any blood vessels. A soft rubber tube is now inserted through an incision in the cecum and is there fixed in place. A second similar tube is inserted into the small intestine to avoid distention and pressure during the experiment, from the accumulation of fluids and gases. The abdominal wall is closed with a few stitches around the rubber tubes. A funnel is inserted into the colon tube for the introduction of fluids. At this stage the large intestine is washed free of feces with warm tap water and finally with warm physiological saline. A soft rubber tube similar to the first is now secured into the rectum of the dog by means of a pursestring suture.

Test fluids may thus be introduced into the upper colon tube and removed from the rectal tube with no manipulation of the intestine. Several tests to determine the accuracy of delivery of fluids, introduced and recovered in this way, show that we are dealing with a maximum error of 2 per cent. The solutions were always introduced warm. The colons of the experimental animals were examined at the conclusion of the experiments and were invariably of normal appearance.

Chloride determinations were made by the Volhard method or by the McLean and Van Slyke (8) method when a limited amount of fluid was available. The latter method was also used for blood chloride determinations. There was never enough protein present to interfere with the Volhard titration; this point was repeatedly controlled. All of the chloride figures are expressed as sodium chloride in the tables below. The depression of the freezing point was determined by means of the Bechmann apparatus. A constant depression below the freezing point was maintained, hence corrections for under-cooling are not made.

In the experiments given below the solutions were introduced in the intestine, allowed to remain for a given interval, removed, volume recorded, and specimens taken for chloride and Δ determinations. In some experiments the same solution was repeatedly removed, in others a different solution was introduced into the intestine.

EXPERIMENTAL.

It is found that when solutions of low concentrations of sodium chloride, or distilled water, are introduced into the colon, there is always a diminution of the volume, the extent of which is dependent upon the length of time the fluid is allowed to remain in the intestine. Upon a comparison of the total amount of sodium chloride introduced (in solutions below 0.16 per cent) with the amount recovered it is found that there is a gain of chlorides in the intestinal contents. As the concentration of the chlorides in the intestinal fluid rises, this gain drops to a low figure and finally shows a decrease in the chlorides, with a greater deficit for each succeeding period thereafter. In other words, it is apparent that up to a certain point chlorides have entered the intestinal contents, followed by a reversal of the process, a passage of chlorides into the blood. The concentration and Δ of each successive solution removed shows a progressively increasing value. Typical protocols are given in table 1.

An exact determination of the concentration and Δ of the point, where the balance of chlorides ceases to be in favor of the gut contents and begins to exhibit a deficit, presents difficulties. It may be either in the last period, showing an excess of chlorides in the intestinal fluid over the amount initially in the solution, or it may equally well be in the first period in which there is a loss of chlorides from the colon contents. Therefore, it can only be said with certainty that the reversing of the phenomenon takes place within a certain range of the concentration and Δ. This range has been placed between the average of the last period of gain of chlorides in the gut and the average of the first period of loss of chlorides from the intestinal fluid. The range is compiled in table 2. An average value is also given of the initial and final concentrations of these two periods. The variation of this average figure, from the individual figures of each experiment is, in every case, less than the physiological variation in different animals.

In order to determine whether this phenomenon is related to the concentration of blood chlorides, observations similar to those above were made before and during intravenous infusion of sodium chloride. Since the kidneys were intact sodium chloride was infused in rather large amounts, slowly, over a long period of time, to insure a definite disturbance of the osmotic equilibrium in the body. Blood chloride determinations show that in each case the blood content of chlorides is appreciably increased. The results are presented in table 3. Under these conditions there is, in each experiment, an increase in the range of concentration of chlorides in which there ceases to be a gain in the gut and a beginning loss of chlorides. These increased values are beyond the physiological variation in an individual animal, hence, we believe, are significant under the experimental conditions. In experiment 69 the range of the control period is from 0.11 to 0.15 per cent, with an average of 0.13 per cent. During the infusion period the values are from 0.24 per cent to 0.29 per cent, with an average of 0.27 per cent. The range of the Δ is also correspondingly higher. Experiment 87 shows a similar behavior. These results, under the conditions described, show that with a large increase of blood chloride content

TABLE 1

The reversible diffusion of chlorides through the intestinal wall

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	CHANGE NaCl: + GAIN, − LOSS	Δ FLUID INTRODUCED	Δ FLUID RECOVERED
	minutes	*cc.*	*cc.*	*per cent*	*per cent*	*mgm.*		
37	30	98	76	0.04	0.11	+44		0.119
	30	90	66	0.05	0.10	+21	0.037	0.109
	30	93	72	0.07	0.12	+21	0.047	0.109
	30	93	72	0.09	0.14	+17	0.065	0.121
	30	92	72	0.11	0.15	+ 7	0.078	0.123
	30	92	75	0.12	0.17	+17	0.085	0.145
	30	92	72	0.14	0.18	0	0.102	0.142
	30	93	76	0.16	0.19	− 4	0.115	0.145
38	30	96	80	0.12	0.15	+12	0.082	0.126
	30	96	78	0.14	0.17	− 2	0.100	0.141
	30	95	77	0.16	0.18	−14	0.111	0.150
	30	96	75	0.18	0.19	−30	0.120	0.157
	30	95	71	0.19	0.20	−39	0.134	0.160
84	30	100	79.5	0.11	0.17	+25	0.094	0.164
	30	100	81	0.13	0.18	+16	0.102	0.161
	30	100	77.5	0.15	0.18	−10	0.112	0.173
	30	100	79.5	0.17	0.19	−19	0.124	0.171
	30	100	77	0.19	0.21	−28	0.131	0.166
87	30	100	79	0.10	0.17	+34	0.061	0.138
	30	100	75	0.11	0.16	+ 9	0.061	0.135
	30	100	76.5	0.13	0.17	0	0.081	0.131
	30	100	75	0.15	0.18	−15	0.091	0.136
	30	100	75	0.17	0.20	−20	0.116	0.162
	30	100	74	0.19	0.21	−35	0.123	0.161

In the following experiments the solution was removed, specimen taken for analysis and remainder returned:

Pantopon 59	30	100	68		0.07	+48		
	30	48	37	0.07	0.11	+ 7		
	20	32	21	0.11	0.13	− 7		0.184
35	60	127	75	0.08	0.17	+26		
	60	55	33	0.17	0.18	−34		
	60	78	40	0.17	0.21	−49		
Decerebrate 78	60	90	75	0.06	0.15	+59		
	60	64.5	49	0.15	0.19	+ 4		
	60	39	25	0.19	0.22	−19		
85	30	140	110	0.08	0.14	+42		
	30	105	82	0.14	0.18	+ 1		
	30	77	57	0.18	0.21	−19		0.205
Pantopon 61	10	132	120	0.10	0.12	+12	0.072	0.102
	10	100	94	0.12	0.14	+12	0.102	
	10	84	79	0.14	0.15	+ 1		0.134
	10	58	54	0.15	0.17	+ 3	0.134	0.142
	10	34	29	0.17	0.19	− 3	0.142	0.174

there is a significant increase in the range and average concentration at which the balance of chloride in the colon contents ceases to be in favor of the intestine. It may be added that in experiment 86, where the volume introduced and the time of sojourn were constant, there is no indication of an increased water loss from the intestinal fluid.

The excess of chloride which has been demonstrated to be present in solutions of very low concentration in the colon, may have its origin in the walls or cells of the intestine, and therefore, represent only a local diffusion from the intestinal wall. If this were the case, it might be expected that when the process has proceeded to a certain point, it would of necessity come to a standstill. Hence, if repeatedly renewed portions of distilled water were brought into contact with the colon, on the above supposition, decreasing amounts of chloride should pass into the fluid at each successive period of observation, and should eventually reach a very low value.

TABLE 2

Chloride threshold. Summary of data

EXPERIMENT NUMBER	AVERAGE CONCENTRATION OF NaCl		AVERAGE CONCENTRATION	AVERAGE Δ		AVERAGE Δ	REMARKS
	Last period of gain	First period of loss		Last period of gain	First period of loss		
	per cent	*per cent*	*per cent*				
35	0.13	0.18	0.16				Same solution repeatedly returned
37	0.16	0.18	0.17	0.122	0.130	0.126	New solution introduced each time
38	0.14	0.16	0.15	0.104	0.121	0.113	New solution introduced each time
49	0.10	0.13	0.12				Same solution repeatedly returned
59(a)	0.09	0.12	0.11				Same solution repeatedly returned
(b)	0.11	0.15	0.13				Same solution repeatedly returned
61	0.16	0.18	0.17	0.138	0.158	0.148	Same solution repeatedly returned
69(a)	0.20	0.23	0.22				Same solution repeatedly returned
(b)	0.11	0.15	0.13	0.100	0.143	0.122	Same solution repeatedly returned
78(a)	0.17	0.21	0.19				Same solution repeatedly returned
(b)	0.11	0.13	0.12	0.114	0.134	0.124	Same solution repeatedly returned
84	0.16	0.17	0.17	0.132	0.143	0.138	New solution introduced each time
85(a)	0.26	0.27	0.27	0.194	0.199	0.197	New solution introduced each time
(b)	0.16	0.20	0.18				Same solution repeatedly returned
87	0.16	0.17	0.17	0.098	0.106	0.102	New solution introduced each time
Average........			0.16			0.134	

TABLE 8

Effect of increased blood chlorides upon the reversible diffusion of chlorides through the intestinal wall

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	CHANGE NaCl: + GAIN − LOSS	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	REMARKS
Control period									
	mins.	*cc.*	*cc.*	*per cent*	*per cent*	*mgm.*			
Pantopon 61	10	132	120	0.10	0.12	+12	0.072	0.102	
	10	100	94	0.12	0.14	+12	0.102		
	10	84	79	0.14	0.15	+ 1		0.134	
	10	59	54	0.15	0.17	+ 3	0.134	0.142	
	10	34	29	0.17	0.19	− 3	0.142	0.174	Blood NaCl = 0.61 per cent
Infused 96 cc. of 10 per cent NaCl intravenously over period of two hours									
	10	141	131	0.10	0.13	+29	0.087		
	10	121	112	0.13	0.15	+11		0.127	
	10	92	83	0.15	0.19	+20	0.127	0.142	
	10	65	60	0.19	0.21	+ 3	0.142		Blood NaCl = 1.1 per cent
	10	50	44	0.21	0.24	0		0.182	
Control period									
69	15	120	100	0.08	0.13	+34	0.074	0.126	Blood NaCl = 0.62 per cent
	20	80	62	0.13	0.16	− 5	0.126	0.160	
	15	45	31	0.16	0.19	−13	0.160	0.202	
Infused slowly 100 cc. of 10 per cent NaCl intravenously during two hours									
	15	118	97	0.09	0.16	+49	0.075	0.128	
	15	80	68	0.16	0.21	+15	0.128	0.185	
	15	50	40	0.21	0.27	+ 3	0.185	0.258 Average 2 periods	Blood NaCl = 0.82 per cent
	15	24	15	0.27	0.30	−20			
Control period									
87	30	100	79	0.10	0.17	+34	0.061	0.138	
	30	100	75	0.11	0.16	+ 9	0.061	0.135	
	30	100	76.5	0.13	0.17	0	0.081	0.131	
	30	100	75	0.15	0.18	−15	0.091	0.136	
	30	100	75	0.17	0.20	−20	0.116	0.162	
	30	100	74	0.19	0.21	−25	0.123	0.161	
	30	100	74	0.22	0.23	−50	0.136	0.191	
	30	100	74	0.27	0.28	−63	0.190	0.218	
Infused 150 cc. of 10 per cent NaCl intravenously during two hours									
	30	100	70	0.19	0.29	+13			
	30	100	75	0.25	0.31	−17			
	30	100	80	0.27	0.33	− 6			

TABLE 4

The diffusion of chlorides into distilled water in colon

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	NaCl RECOVERED	GAIN IN NaCl TO GUT CONTENTS	Δ FLUID RECOVERED	REMARKS
	mins.	*cc.*	*cc.*	*per cent*	*mgm.*		
37	30	100	89	0.18	160	0.189	
	30	100	84	0.15	126	0.162	
	30	100	89	0.12	107	0.115	
	30	100	79	0.11	87	0.110	Total NaCl = 0.480 gram
38	30	100	83	0.10	82	0.103	
	30	100	83	0.07	58	0.080	
	30	100	80	0.08	64	0.084	
	30	100	80	0.06	48	0.065	Total NaCl = 0.252 gram
54	30	93	77	0.08	62		Thymol added to contents in colon for other purposes
	30	100	72	0.07	50		
	30	100	77	0.06	46		
	30	100	70	0.06	42		
	30	100	71	0.05	36		
	30	100	73	0.05	37		
	30	100	59	0.09	53		Total NaCl = 0.326 gram
55	60	96	65	0.17	110		
	60	100	62	0.13	81		
	60	100	56	0.10	56		
	60	100	56	0.08	45		Total NaCl = 0.292 gram
42	60	100	70	0.19	136	0.219	At beginning of experiment Blood Δ = 0.604 Blood NaCl = 0.62 per cent
	60	100	72	0.14	101	0.160	
	60	100	69	0.10	69	0.211	
	60	100	65	0.10	65	0.120	
	60	100	69	0.10	69	0.110	At end of experiment Blood Δ = 0.554
	60	100	68	0.10	68	0.121	Blood NaCl = 0.54 per cent
	60	100	71	0.08	58	0.099	Total NaCl = 0.566 gram
		Effect of increased blood chlorides					
28	60	100	64	0.08	51		At beginning of experiment Blood NaCl = 0.59 per cent
	60	100	59	0.07	41		
	60	100	63	0.07	44		
	60	100	60	0.07	42		
		Infused 158 cc. of 10 per cent NaCl during 1 hour 20 minutes					
	60	100	65	0.08	52		
	60	100	66	0.14	92		
	60	100	65	0.13	85		At end of experiment Blood NaCl = 0.88 per cent Total NaCl = 0.407 gram

Experiments designed to test this point are given in table 4. There is no evidence from these and other figures which are available, that we are dealing with a very limited source of chlorides. In the first periods the values may be somewhat higher than in the succeeding periods; however, this excess might very well arise from the fluids bathing the intestine. The subsequent values are significantly high. The total amount of chlorides withdrawn in each experiment is also calculated, and give values from 0.250 to 0.590 gram of sodium chloride. The total amount of chlorides present in the intestine is a function of the time which the fluid is allowed to remain in the gut. Comparisons of half and one hour experiments are made. That the continued withdrawal of chlorides through the intestine may affect the blood chlorides and the blood Δ is shown in experiment 42. A withdrawal of 0.556 gram of sodium chloride from this animal, resulted in a decrease of blood chlorides as sodium chloride from 0.62 per cent to 0.54 per cent, with a decrease in the blood Δ from 0.604 to 0.554.

TABLE 5

Diffusion of chlorides into distilled water in colon of dead animal

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME (−LOSS)	NaCl RECOVERED	CHANGE NaCl: −LOSS+GAIN	REMARKS
	mins.	*cc.*	*cc.*	*cc.*	*per cent*	*mgm.*	
31	30	90	87	−3	0.02	+17	Intestine in situ
	30	90	88	−2	0.02	+18	
	30	90	89	−1	0.02	+19	
	30	90	90	0	0.01	+ 9	
32	60	90	90	0	0.09	+81	Intestine removed from body
	60	90	87	−3	0.04	+35	
	75	90	87	−3	0.02	+17	

Experiment 28, at the end of table 4, demonstrates the effect of intravenous infusion of sodium chloride upon the passage of chlorides into distilled water in the colon. A graphic representation is also given in figure 1, where the total amount of chlorides (as sodium chloride) diffusing into the intestine is represented. The time and volume of fluid are constant in this experiment. It is quite evident that an increase of blood chlorides, under these conditions, causes an increase in the concentration and total amount of chlorides in the intestinal fluid. An increased absorption of water from the intestine is not demonstrable; the values before and during infusion are of the same order.

In this connection it seemed of interest to determine the changes occurring in distilled water in the intestine of dead animals. In experiment 31, (table 4) the intestine was allowed to remain in situ; in experiment 32 it was removed from the body and kept moist throughout the experiment.

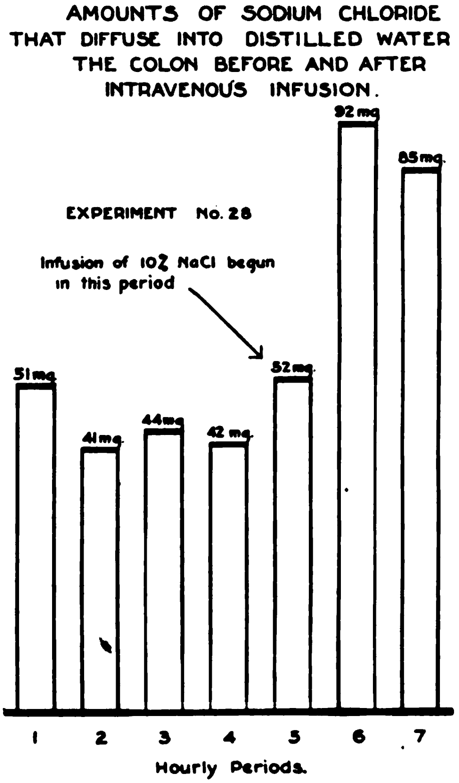

Fig. 1

In contrast to the former experiments, the distilled water in the intestine of a dead animal shows little or no tendency to disappear. Furthermore, the concentration of chlorides and the total amount present is much less than in the experiments on living animals, and decreases rapidly with each successive period. The total amount of chlorides withdrawn might well have its origin in the walls and cells of the intestine. This, also, points to the fact that the ultimate origin of the chlorides, withdrawn in our experiments on living animals, is the blood.

DISCUSSION

The experimental work presented herewith demonstrates that up to the point where the intestinal contents reach a certain concentration, chlorides may pass into the colon. The approximate value of this concentration is 0.16 per cent, with a Δ of 0.134. It is evident that we are dealing here with a "factor of safety" in the form of a threshold value which has its analogy in the kidney threshold. It is the result of a mechanism which tends to prevent the loss of large amounts of body salt through the intestine. That this mechanism stands in relation to the blood content of salt, at least in part, has been demonstrated. A comparison of these results with those obtained by Cohnheim (1) and Waymouth-Reid (2) is not possible, inasmuch as their work was done upon the small intestine. Nevertheless, it is evident from the results presented that the colon is not characterized by a strictly one-sided permeability. The failure of these authors to demonstrate large amounts of sodium chloride in the intestinal contents may have its explanation in our threshold value. Our results also show why, as in Heidenhain's (4) experiments, an excess of sodium chloride cannot be demonstrated in solutions of 0.3 to 0.5 per cent.

We cannot believe with Waymouth-Reid (2) that this influx of sodium chloride into distilled water or into solutions below 0.16 per cent is due to an injury to the wall of the colon. Physiologically, there is no evidence of injury. The ultimate behavior of distilled water, or of a 0.2 per cent or a 0.5 per cent sodium chloride solution in the colon, is the same. Water is absorbed, and the concentration and Δ rise with all three solutions; the only difference to be found is in the fact that chlorides may pass into the distilled water, whereas at the higher concentrations there is a loss of chloride. We have repeatedly, in the course of our work, introduced distilled water into the colon, and subsequently tested the behavior of other solutions. A qualitative or quantitative difference in the behavior after distilled water or physiological saline has never been noted.

Our results are in accord with those of Waymouth-Reid (2) that there is not an increased water absorption from the intestine when the blood chloride content is raised by intravenous infusion of sodium chloride. However, we have shown that an increased amount of chlorides passes into distilled water, with a rise in the threshold, under these conditions.

Two protocols in the paper by Diena (6) show a positive balance of sodium chloride in the intestine, after a solution of 0.19 per cent sodium chloride is allowed to remain in the colon for two hours. The final concentrations are, respectively, 0.4 and 0.475 per cent. In none of our experiments, under the most extreme conditions, (intraveous infusion of sodium chloride), have we ever found a positive balance of chlorides at a final concentration of 0.4 per cent. We wish to suggest that this difference is due to a difference in the experimental methods employed. At the end of the period of observation Diena washes the intestine with distilled water (270 to 300 cc.). The chlorides thus withdrawn are added to the total chlorides recovered. Our experiments show that chlorides pass rapidly into distilled water in the colon. Experiments have been performed in which distilled water was merely passed through the intestine, and we find that considerable amounts of chlorides may be thus abstracted. The chlorides of the wash fluid, in each of Diena's experiments, are greater than the excess of chlorides found by him. Hence, disregarding the amount of chlorides recovered in this fashion, his experiments show a loss of chlorides from the colon. We are in accord with Diena's conclusion that chlorides pass into strongly hypotonic solutions in the colon, but cannot agree that this occurs at the sodium chloride concentrations of the final fluids withdrawn by him.

CONCLUSIONS

1. The colon is not characterized by a strictly one-sided permeability.
2. There is a threshold of the colon, below which chlorides diffuse from the blood stream into the intestinal contents.
3. The blood content of chlorides is a factor in determining the height of this threshold.
4. The chlorides diffusing into distilled water or very low concentrations of sodium chloride in the colon have their origin in the blood.

BIBLIOGRAPHY

(1) Cohnheim: Zeitschr. f. Biol., 1897, xxxvi, 129; 1899, xxxvii, 443; 1900, xxxix, 167.
(2) Waymouth-Reid: Phil. Trans. Roy. Soc. London, 1900, cxcii, 211.
(3) Waymouth-Reid: Journ. Physiol., 1902, xxviii, 241.

(4) Heidenhain: Pflüger's Arch., 1894, lvi, 579.
(5) Hamburger: Arch. f. Anat. u. Physiol., 1896, 36; Biochem. Zeitschr., 1908, xi, 443.
(6) Diena: Arch. Sci. Mediche, 1911, xxxv, 63.
(7) Waymouth-Reid: Phil. Trans. Roy. Soc. London, 1900, cxcii, 211.
(8) McLean and Van Slyke: Journ. Biol. Chem., 1915, xxi, 361.

STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

II. The Colon. On the Passage of Fluid in Two Directions through the Intestinal Wall[1]

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It has been shown (1) that there is a threshold value above which loss of chlorides from the blood into the intestinal fluid is inhibited, under ordinary conditions. It seemed of interest, therefore, to determine whether a similar "factor of safety" controlled water loss from the blood into the intestine.

Heidenhain (2) finds that water may be absorbed from solutions of sodium chloride of various concentrations in the small intestine, that is from a 1.46 per cent as well as from a 2.0 per cent solution. He considers that the fluid absorption in these cases is a function of the "physiological activity" of the intestinal epithelium. Since the osmotic pressure of the solution is greater than that of the blood the osmotic factors are not concerned in the process. Although the "physiological activity" is capable of varying in value, the author states that the border-line concentration at which it is overcome is at 2.0 per cent of sodium chloride. Above this point osmotic pressure factors come into play and fluid passes into the intestine, but the author states that solutions of sodium chloride above 2.0 per cent may exert an injurious effect upon the intestinal epithelium and thus modify the absorption phenomenon.

On the basis of investigations upon the isolated small intestine of cats, rabbits and dogs, Cohnheim (3) believes to have demonstrated an active transport of fluid, inasmuch as there is a loss of fluid from the intestine with no increase of weight of the intestine. This transport in one direction, toward the blood, has its origin in the cells of the intestinal wall.

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[1] A preliminary report was made before The American Society of Biological Chemists, December, 1916. Journ. Biol. Chem., 1917, xxix, p. xii.

Waymouth-Reid (4) confirms the observation of Cohnheim that a secretory transport of fluid is capable of demonstration in the isolated small intestine of cats. This takes place in all instances from the intestinal lumen.

We well realize that the behavior of the fluid of solutions introduced into the intestine depends upon the nature of the solute, as Wallace and Cushny (5) and Höber (6) have shown. The intestinal wall is readily permeable to sodium chloride, hence the results of the present investigation would apply only to salts of this class.

The experimental methods are similar in all respects to those used in the previous paper (1). Since the volume of fluid remains sufficiently large, the same solutions are repeatedly returned in all of the experiments given below.

EXPERIMENTAL

In the typical protocols, given in table 1, it is shown that when solutions of sodium chloride (above 1.2 per cent) of osmotic pressure sufficiently higher than the blood, are introduced into the colon of dogs, fluid passes into the intestinal contents while chlorides pass into the blood. The effect is a rapid reduction in the sodium chloride concentration and osmotic pressure of the intestinal contents. Figure 1 gives a graphic representation of these volume and concentration changes in a typical experiment.

In determining the concentration of sodium chloride and the Δ at which the fluid balance ceases to be positive in the intestine, and fluid begins to leave, we are confronted with the same difficulties as in the determination of the salt threshold in our previous paper. The turning point may be either in the last period which shows a gain of fluid to the gut, or in the first period which exhibits a loss of fluid.

Hence in table 2 has been compiled the average concentration of sodium chloride in each of these periods. This gives a range between which the point must lie. An average of the range of each experiment has also been noted. This gives an approximate value for the concentration of chlorides, at the point where the fluid content of the colon turns from a positive to a negative balance. The average Δ has been computed similarly, thus an approximate value of sodium chloride concentration of 1.21 per cent with a Δ of 0.786 is obtained. It will be seen that the variations in the individual experiments are not great,

TABLE 1

The reversible diffusion of fluid through the intestinal wall

EXPERIMENT NO.	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME − Loss, + GAIN	NaCl INTRODUCED	NaCl RECOVERED	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	CHANGE NaCl IN GUT
	min.	*cc.*	*cc.*		*per cent*	*per cent*			*mgm.*
17	60	28	43	+15	4.22	1.56			−511
	60	42	47	+ 5	1.56	1.06			−157
	60	46	36	−10	1.06	0.81			−196
	60	35	17	−18	0.81	0.64			−175
29a	30	100	113	+13	1.99	1.55			−240
	30	109	118	+ 9	1.55	1.33			−120
	30	114	112	− 2	1.35	1.15			−228
	30	108	101	− 7	1.15	1.05			−182
29b	30	95	102	+ 7	1.45	1.19		0.821	−164
	30	88	85	− 3	1.19	1.07	0.821	0.721	−138
	30	71	65	− 6	1.07	0.96	0.721	0.662	−136
	30	51	41	−10	0.96	0.90	0.662	0.670	−121
30	30	86	103	+17	1.57	1.35	0.987	0.878	
	30	89	94	+ 5	1.35	1.20	0.878	0.795	− 74
	30	80	78	− 2	1.20	1.07	0.795	0.702	−125
	30	64	63	− 1	1.07	1.00	0.702	0.671	− 55
	30	49	41	− 8	1.00	0.90	0.671	0.626	−130
33	30	131	139	+ 8	1.64	1.39	1.009	0.901	−216
	30	125	134	+ 9	1.39	1.23	0.901	0.820	− 89
	30	120	125	+ 5	1.23	1.13	0.820	0.745	− 64
	30	111	109	− 2	1.13	1.00	0.745	0.680	−164
	30	95	86	− 9	1.00	0.94	0.680	0.674	−142
	30	72	64	− 8	0.94	0.88	0.674	0.660	−114
	30	50	40	−10	0.88	0.84	0.660	0.630	−104
34	30	134	134	0	1.65	1.48	1.005	0.930	−228
	30	120	122	+ 2	1.48	1.33	0.930	0.838	−153
	30	108	118	+10	1.33	1.19	0.838	0.771	− 32
	30	104	102	− 2	1.19	0.07	0.771	0.702	−146
	30	88	83	− 5	1 07	0.97	0.702	0.676	−137
	30	69	62	− 7	0.97	0.89			−118
	30	48	41	− 7	0.89	0.85		0.603	− 79
Decerebrate 60	10	100	112	+12	1.90		1.200		
	15	112	116	+ 4		1.48		0.934	−184
	15	100	102	+ 2	1.48		0.934		
	15	102	102	0		1.24		0.838	−215
	15	85	80	− 5	1.24	1.20	0.838	0.827	− 94
	15	65	58	− 7	1.20				
	15	58	53	− 5		1.09			−202
	20	49	38	−11	1 09	1.04		0.743	−139

TABLE 1—*Continued*

EXPERIMENT NO.	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME — LOSS, + GAIN	NaCl INTRODUCED	NaCl RECOVERED	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	CHANGE NaCl IN GUT
	min.	*cc.*	*cc.*		*per cent*	*per cent*			*mgm.*
Decerebrate 74	15	100	107	+ 7	1.96		1 96		
	15	107	115	+ 8		1.48		0.917	−260
	15	101	103	+ 2	1.48		0.917		
	15	103	104	+ 1		1.21		0.781	−236
	15	90	88	− 2	1.21	1.11	0.781	0.737	−112
	15	75	70	− 5	1.11	1.05	0.737	0.686	− 98
	30	57	50	− 7	1.05	0.96	0.686	0.646	−119
Effect of NaCl infusion (intravenously) upon "crest" of volume curve									
65	15	146	149	+ 3	1.91		1.180		
	15	149	158	+ 9		1.64		1.159	−197
	15	144	151	+ 7	1.64		1.159		
	15	151	157	+ 6		1.47		0.949	− 54
	15	143	147	+ 4	1.47	1.40	0.949	0.921	− 44
	15	133	139	+ 6	1.40	1.30	0.921	0.851	− 55
	15	125	128	+ 3	1.30	1.24	0.851	0.821	− 38
	15	108	110	+ 2	1.24	1.17	0.821	0.774	− 52
	15	90	92	+ 2	1.17	1.11	0.774	0.741	− 32
	15	72	71	− 1	1.11	1.03	0.741	0.697	− 68
	15	51	50	− 1	1.03	0.97	0.697		− 40
	35	46	42	− 4	0.97	0.85		0.610	− 89
Infused intravenously 116 cc. of 10 per cent NaCl over period of four hours									
	30	90	89	− 1	1.46	1 41			
	30	85	81	− 4	1.41	1.38		0.892	
Pantopon 59	15	90	90	0	1.44		0.955		
	15	90	94	+ 4		1.10		0.744	−262
	15	80	75	− 5	1.10	0.98	0.744	0.705	−145
	20	60	54	− 6	0.98	0.87	0.705	0.656	−118

and differ but slightly from the average. This is the more striking when one considers that physiological variations as well as experimental errors are involved.

In table 1 has been added a single protocol showing the effect of increased blood chloride content upon this phenomenon. It will be seen that, whereas in the normal period there is a gain of fluid (4 cc.) to the gut at a concentration of sodium chloride of 1.47 to 1.40 per cent, at a corresponding concentration, during infusion, there is prac-

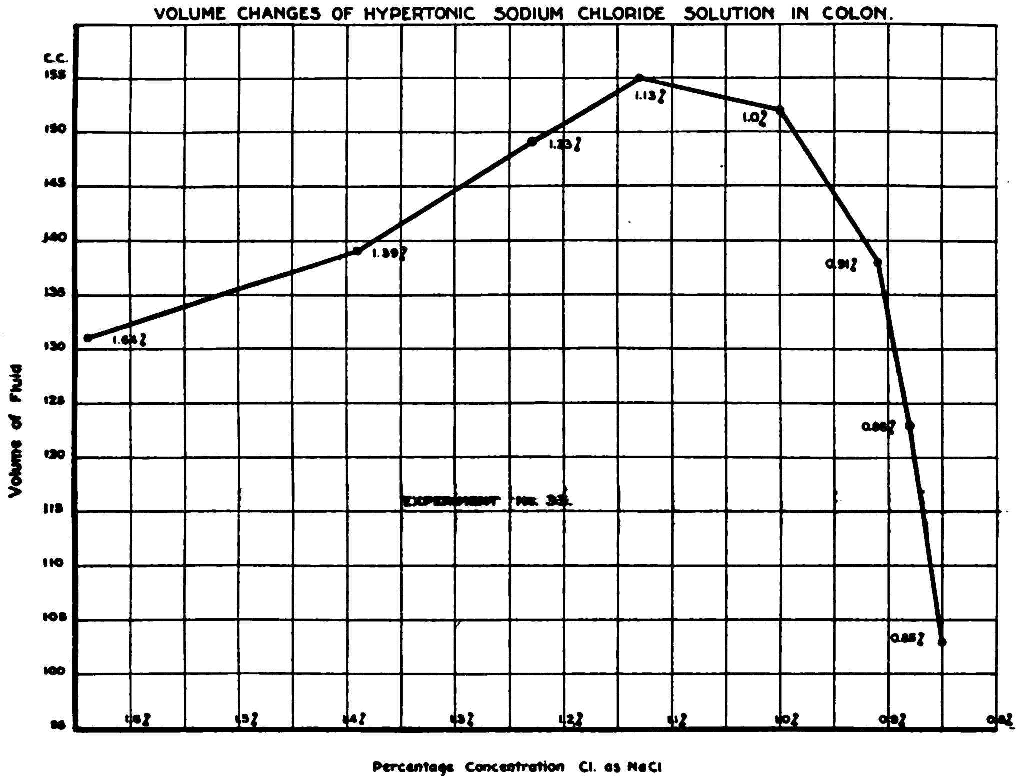

Fig. 1

TABLE 2

Fluid threshold. Summary of data

EXPERIMENT NUMBER	AVERAGE CONCENTRATION OF LAST AND FIRST PERIODS RESPECTIVELY, IN WHICH GUT CONTENTS SHOW		AVERAGE CONCENTRATION	AVERAGE Δ OF LAST AND FIRST PERIODS RESPECTIVELY, IN WHICH GUT CONTENTS SHOW		AVERAGE Δ
	Gain in volume	Loss in volume		Gain in volume	Loss in volume	
	per cent	*per cent*	*per cent*	Δ	Δ	Δ
17	1.31	0.94	1.13	—	—	—
29(a)	1.44	1.24	1.34			
29(b)	1.32	1.13	1.23			
30	1.28	1.14	1.21	0.839	0.749	0.793
33	1.18	1.07	1.13	0.783	0.713	0.748
34	1.26	1.13	1.20	0.805	0.737	0.771
59	1.27	1.04	1.16	0.850	0.725	0.788
60	1.36	1.22	1.29	0.886	0.833	0.860
61	1.14	1.07	1.11	0.758	0.719	0.739
74	1.35	1.17	1.26	0.849	0.759	0.804
Average........................			1.21			0.786

tically no fluid interchange (a loss of 1 cc.), the tendency as indicated by the figures being toward a loss of fluid from the colon. In the normal period the range of concentration in which the turning point occurred is from 1.14 to 1.07 per cent. In the infusion period it is but slightly below 1.46 to 1.41 per cent. This shows that the concentration of chlorides at which the turning point occurs has been raised.

DISCUSSION

The results of the experiments detailed above lead us to believe that, just as we have found that there is a threshold for the passage of sodium chloride out of the blood into the intestine, there is a similar mechanism which controls the loss of fluid from the blood into the intestine. One part of this mechanism is apparent from the results obtained in these experiments, namely, a rapid absorption of sodium chloride or the absorption of the chlorides in a very concentrated solution, against an inflow of fluid into the intestine.

We might expect that this combined effect would continue, as Heidenhain (2) pointed out, until the solution in the intestine becomes isotonic with the blood. On the contrary, the water inflow ceases at a concentration of sodium chloride and at an osmotic pressure of the colon contents considerably above the osmotic pressure of the blood. The chloride absorption continues rapidly to reduce the osmotic pressure of the solution in the colon. Hence, although we are in agreement with Heidenhain's (2) conclusion that solutions of sodium chloride of higher osmotic pressure than the blood may exhibit fluid absorption in the intestine, the concentration at which fluid passes into the colon contents (approximately 1.21 per cent) is much lower than that found by Heidenhain in the small intestine, namely 2.0 per cent.

It will, moreover, be noted that in our experiments the same results are obtained with a sodium chloride concentration of 4.22 per cent, experiment 17, as with a solution of 1.45 per cent, experiment 29b. The difference in concentration of these two solutions is so great that we cannot but believe that if, as Heidenhain (2) suggested, strong solutions of sodium chloride injure the intestinal wall, there would be an evident difference of effect in these two experiments.

Cohnheim (3) and Waymouth-Reid (4) have attributed the fluid absorption in the intestine to an "active transport," which is a function of the epithelial cells of the intestine. We have shown that an increase of blood chlorides, by intravenous infusion of sodium chloride, raises the water threshold. Therefore, this "active transport" may be altered from the blood side, hence we cannot affirm the view held by these authors that it is entirely a function of the intestinal epithelium.

CONCLUSIONS

1. Hypertonic solutions of sodium chloride which readily pass through the intestinal wall, attract fluid into the intestine above a certain threshold value, approximately 1.20 per cent with a Δ of 0.786.
2. At the same time chlorides pass in concentrated solutions into the blood.
3. The concentration of the solute (sodium chloride) and the Δ at the threshold point, that is the point at which the fluid balance just ceases to be in favor of the colon, shows a striking constancy in a long series of experiments.
4. There is evidence that increased blood chlorides may effect this threshold value.

BIBLIOGRAPHY

(1) Goldschmidt and Dayton: This Journal, 1919, xlviii, 419.
(2) Heidenhain: Pflüger's Arch., 1894, lvi, 579.
(3) Cohnheim: Zeitschr. f. Biol., 1899, xxxviii, 419.
(4) Waymouth-Reid: Journ. Physiol., 1901, xxvi, 436.
(5) Wallace and Cushny: This Journal, 1898, i, 411.
(6) Höber: Pflüger's Arch., 1898, lxx, 624.

STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

III. THE COLON. THE OSMOTIC PRESSURE EQUILIBRIUM BETWEEN THE INTESTINAL CONTENTS AND THE BLOOD

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A further link in the comparison of the passage of solute and solvent through the intestine, with diffusion through homogeneous membranes is a study of the partial pressure equilibrium of diffusible dissolved substances and the total osmotic equilibrium. An investigation of these points is presented in this paper.

Gumilewsky (1), using animals with a Thiry-Vella fistula of the small intestine, finds that from solutions of sodium chloride of a concentration of 0.6 per cent the water and salt are absorbed at the same rate, hence the concentration is not changed. From solutions of 0.25 per cent sodium chloride, the author finds that water is absorbed faster than the salt, whereas the process is reversed from 1.0 per cent solutions.

Heidenhain (2) makes the same observation in regard to solutions of 0.6 per cent sodium chloride.

Waymouth-Reid (3) finds that glucose, and mixtures of glucose and sodium chloride, can be chosen which neither increase nor decrease in concentration.

Höber (4) states that when solutions, isotonic with the blood serum, are subjected to intestinal absorption, the osmotic pressure of the solution during absorption does not remain unchanged but is occasionally raised.

Dieña (5), using the permanently isolated colon of dogs, investigated the behavior of solutions of sodium chloride at various concentrations. This author finds a large quantity of water is absorbed from hypotonic solutions and that this absorption decreases with increasing concentration. At isotonicity there is very little fluid absorption. This investigator compares the observed Δ of solutions of various concentrations of sodium chloride, after a period of sojourn in the colon, with the theoretical Δ of pure solutions of the same sodium chloride content. It is observed that the osmotic pressure of the solution is always higher than can be accounted for by the sodium chloride content of the solution. The conclusion is therefore drawn that osmotically active substances pass from the organism into the liquid, regardless of the osmotic pressure of the fluid in the colon. Furthermore, Diena states that the osmotic pressure of the intestinal fluid in all concentrations approaches that of the blood.

Hamburger (6) observes, in the peritoneal and pericardial cavities of rabbits, that hypertonic and hypotonic solutions of various salts (including NaCl) come into osmotic equilibrium with the blood, while isotonic solutions show no change in osmotic pressure.

Leathes and Starling, (7), in an investigation into the mechanism of absorption from the pleural cavity, find that absorption of sodium chloride proceeds according to the partial pressure of the salt.

The experimental methods in this investigation are the same as used in previous experiments of this series (8). All of the work is confined to the colon of dogs. Blood chlorides were determined by the method of McLean and Van Slyke. The blood Δ was determined on the whole blood.

EXPERIMENTAL

In table 1 are tabulated several typical protocols which demonstrate that, if a solution of sodium chloride above (exper. 24, etc.) or below (exper. 25) blood level is introduced into the colon, the concentration approaches that of the blood. This is graphically represented in figure 1. In the low concentrations the solvent passes faster than the solute, in concentrations above the blood content, the solute faster than the solvent.. With solutions below blood level, the calculated concentrations of the chlorides in the absorbed fluid show increasing values (exper. 25), approaching the concentration of the solution in the intestine as partial pressure equilibrium is established. On the other hand, with solutions of sodium chloride above blood level, decreasing values are obtained (exper. 24) which approach the same level.

Occasionally, as in experiments 26, 29 and 74, the intestinal contents apparently come into equilibrium at a concentration above the con-

TABLE 1

Partial pressure equilibrium between blood and intestinal chlorides

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	NaCl BLOOD	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	Δ BLOOD	CONCENTRATION ABSORBED NaCl	REMARKS
	minutes	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*					
23	60	93	64	0.93	0.85					0.94	Contents returned
	60	62	39	0.85	0.83					0.88	Contents returned
	60	37	18	0.83	0.77					0.89	Contents returned
	60	103	50	0.76	0.73					0.79	Fresh solution
	60	48	17	0.73	0.68					0.75	Contents returned
24	60	82	65	0.85	0.77					1.15	Contents were made up to volume with solutions of equal concentration
	60	90	60	0.77	0.72					0.87	
	60	73	54	0.72	0.68					0.83	
	60	73	40	0.67	0.64					0.71	
	60	81	29	0.64	0.62					0.65	
	60		18	0.63	0.62						
	60	76	21	0.61	0.62	0.59				0.60	
25	60	91	63	0.33	0.41	0.66				0.15	Contents were made up to volume with solutions of equal concentration
	60	88	64	0.41	0.45					0.30	
	60	88	54	0.45	0.49					0.45	
	60	92	53	0.48	0.50					0.45	
	60	91	44	0.50	0.54					0.46	
	60	89	48	0.54	0.57	0.61				0.51	
26	60	92	59	0.63	0.69	0.62				0.52	Contents were made up to volume with solutions of equal concentration
	60	89	57	0.68	0.70					0.64	
	60	94	62	0.69	0.71					0.65	
	60	91	59	0.72	0.72	0.64				0.72	
29	60	100	52	0.64	0.74			0.535		0.53	Contents were made up to volume with solutions of equal concentration
	60	96	50	0.75	0.75	0.66		0.575		0.75	
35	60	86	35	0.67	0.67		0.443	0.500		0.67	
	60	86		0.65	0.67		0.428	0.501	0.573		Fresh solution
36	60	136	111	0.65	0.68		0.445	0.480		0.52	
	60	97	76	0.68	0.70		0.480	0.520		0.61	Contents returned
	60	62	35	0.70	0.68	0.67	0.520	0.510	0.565	0.73	Contents returned
	60	82		0.68	0.67		0.441	0.480			Fresh solution
	60	186	140	0.88	0.84		0.580	0.565		1.00	Fresh solution
	60	126	100	0.84	0.77		0.565	0.550		1.10	Contents returned
	60	86	58	0.77	0.74		0.550	0.550	0.563	0.83	Contents returned

TABLE 1—*Continued*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	NaCl BLOOD	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	Δ BLOOD	CONCENTRATION ABSORBED NaCl	REMARKS
	minutes	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*					
74	60	95	59	0.76	0.75		0.465	0.527		0.78	
	30	40		0.75	0.73		0.527	0.559			Contents returned
	60	95	42	0.51	0.63		0.322	0.446		0.42	Fresh solution
	60	100	47.5	0.66	0.70		0.416	0.476		0.63	Fresh solution
	30	99	67	0.71	0.72		0.434	0.478		0.71	Fresh solution
	30	48	28	0.72	0.72	0.64	0.478	0.506		0.72	Contents returned
Effect of spontaneous increase of blood chlorides upon chloride equilibrium											
30	30	93	63	0.80	0.79			0.554		0.82	Contents made up to volume
	30	90	62	0.78	0.78			0.556		0.78	
	30	96	49	0.60	0.71	0.73		0.552		0.49	Fresh solution High blood Cls due to anuria
33	60	178	128	0.81	0.79		0.545	0.556		0.86	
	60	114	73	0.79	0.76		0.556	0.567		0.84	Contents returned
	60	59	33	0.76	0.76	0.80	0.567	0.567		0.76	High blood Cls due to anuria
Effect of intravenous infusion of NaCl upon chloride equilibrium											
27	60	41	21	0.64	0.70	0.65				0.57	
	60	38	13	0.70	0.69					0.70	Fresh solution
	60	42	11	0.69	0.66					0.70	Fresh solution
	60	38	8	0.65	0.64					0.63	Fresh solution
	60	60	23	0.63	0.66					0.61	Fresh solution
	60	61	27	0.63	0.66	0.65				0.61	Fresh solution
Infused intravenous, 144 cc. 10 per cent NaCl over period 3 hours 40 minutes											
	60	63	32	0.64	0.70					0.58	
	60	64	28	0.68	0.78					0.60	Fresh solution
	60	59	52	0.75	0.88	0.97				—	Fresh solution
Behavior in dead animal											
							Change NaCl −loss +gain				
31	60	81	81	0.75	0.72		−24 mgm.				Solution returned each time
	60	71	69	0.72	0.72		−14				Solution returned each time
	60	59	60	0.72	0.73		+13				Solution returned each time

centration of blood chlorides, or as in experiments 36 and 74, especially when fresh solutions at a concentration close to blood level are introduced, the concentration of the intestinal chlorides may rise above that of the blood. These points will be discussed later in the paper.

The Δ of the solution shows an interesting behavior. In experiment 33 the Δ comes to a standstill at 0.567 with a concentration of chlorides of 0.76 per cent. On the other hand, in the first periods of experiments 35 and 74, although there is no change of concentration of chlorides, the Δ rises. In the last periods of experiment 36, and the second period of 74, in spite of the fact, that the concentration of chlorides decreases, the Δ rises.

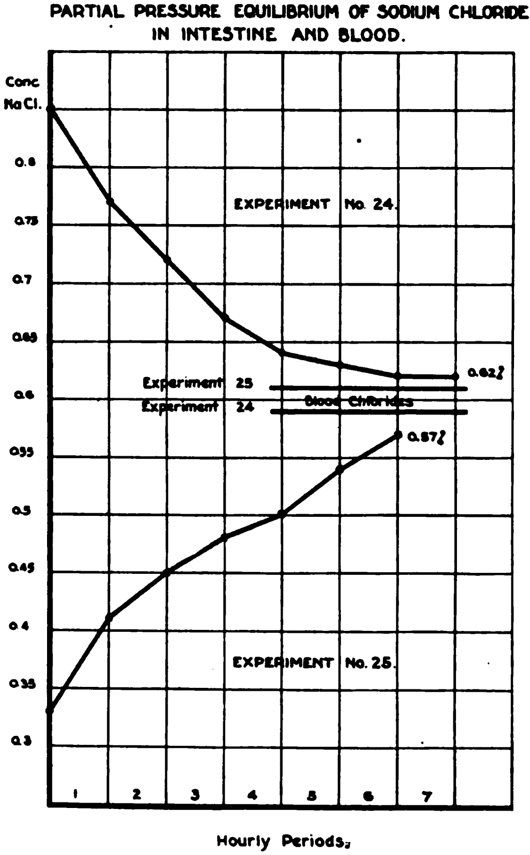

Fig. 1

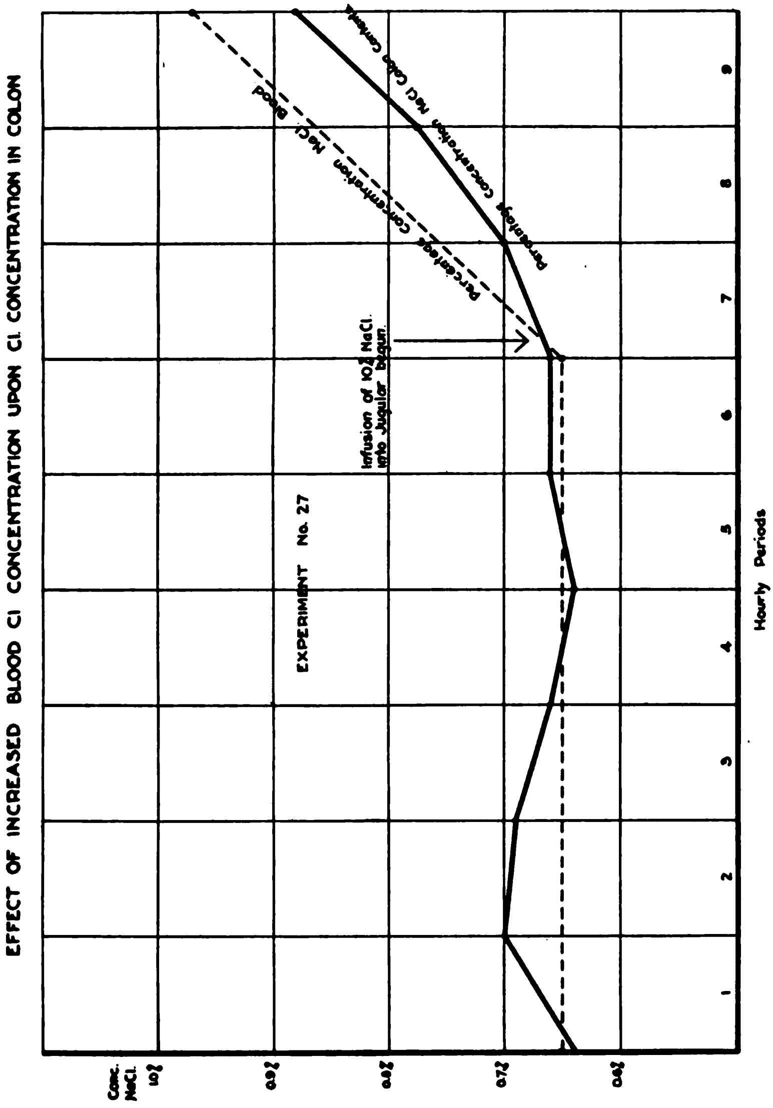
EFFECT OF INCREASED BLOOD Cl CONCENTRATION UPON Cl CONCENTRATION IN COLON
EXPERIMENT No. 27
Percentage Concentration NaCl Colon Contents
Percentage Concentration NaCl Blood
Infusion of 10% NaCl into Jugular begun.
Conc. NaCl
1.0%
0.9%
0.8%
0.7%
0.6%
1
2
3
4
5
6
7
8
9
Hourly Periods

Fig. 2

In the table are given two protocols of experiments in which, early in the period of experimentation, the dogs developed a spontaneous anuria, the result of which was an increase of the blood chlorides. This increase was no doubt in part due to the fact that a considerable amount of chloride was being absorbed from the colon. In experiment 30 the blood rises to 0.73 per cent sodium chloride and in experiment 33 to 0.80 per cent. In each case the concentration of sodium chloride in the gut rises to a comparatively high figure. We merely wish to suggest this as a probable cause and effect in these two experiments for, as we pointed out above in experiments 26, 29 and 74, with normal blood chlorides, there was an apparent equilibrium above blood level.

Experiment 27 shows, definitely, that on raising the blood chlorides by intravenous infusion of sodium chloride a new point of equilibrium between the intestinal contents and the blood is established. A 0.75 per cent solution of sodium chloride introduced during the infusion rises to 0.88 per cent when the blood content is 0.97 per cent. In the normal animal an equilibrium is obtained at 0.66 per cent sodium chloride in the gut with a 0.65 per cent blood content. This is represented in figure 2.

At the end of the table is a typical protocol showing the behavior of solutions of sodium chloride at about blood level, in the colon of a dead animal. The volume of fluid shows little or no change from hour to hour. The same is true of the chloride concentration.

DISCUSSION

The results of the experimental work given above demonstrate conclusively that when solutions of sodium chloride, of greater or less concentration than the blood chloride content, are introduced into the intestine, they come into a partial osmotic pressure equilibrium with the blood. This is in accord with the observations of Gumilewsky (1) and Heidenhain (2) that solutions of sodium chloride of about 0.6 per cent exhibit little change in concentration when introduced into the small intestine. In the two experiments reported by Diena (5) in which 0.92 per cent sodium chloride is introduced into the isolated colon of a dog, there is a decrease in the concentration of the chlorides in the colon content to about blood level, although the author does not mention this point. Our results are in harmony with those of this investigator.

That this equilibrium is related to the blood is further shown by the behavior of gut chlorides, when the blood chlorides spontaneously rise due to an absorption of chlorides and a diminished excretion through the kidney. That the gut chloride content is dependent upon the concentration of chlorides in the blood, is definitely proven by the establishment of a higher level of equilibrium when the blood chlorides are experimentally increased.

In the dead animals the fluid and chloride absorption show very slight or negative values, hence there is a mere stasis of fluid in the intestine in contrast to an actual interchange in the living animal.

In figure 3 is plotted the observed Δ in the solutions from the colon, superimposed upon the Δ of solutions containing the same concentration of sodium chloride. The latter solutions were made with the same sodium chloride used in the experiments and the Δ determined as with the experimental solutions. The two plots show a wide divergence. The reason for the divergence at the start is the fact that solutions used in our experiments are always first rinsed through the intestine before the sodium chloride content is determined. By this procedure, any dilution from fluid in the colon or any substances which would effect the Δ of the initial solution is taken into account. We can only explain this later divergence, of observed Δ and the Δ determined from actual amount of sodium chloride in the intestinal fluid, on the basis that substances other than chloride, producing osmotic pressure, are present in the gut fluid. Hence, the Δ shows higher values than can be accounted for by the sodium chloride alone. A sysatic determination of these constituents has not been made. Diena (5) reports finding sodium chloride and nitrogenous substances diffusing into glucose solutions in the colon.

Our results are in accord with those of Diena (5), in showing that there is an attempt at a total osmotic equilibrium between the colon contents and the blood. Hamburger (6) showed a similar behavior in the peritoneal and pericardial cavities, and Leathes and Starling (7) in the pleural cavity.

We have tactily assumed in this comparison of intestinal chloride content with equivalent solutions of sodium chloride, that the chlorides estimated in the intestinal contents exist as sodium chloride. However, no other molecular combination, physiologically possible, can adversely affect the conclusion.

We have observed, as mentioned above, first: an apparent equilibrium at a concentration of chlorides above blood level; second: an increase of the Δ of the solutions with little or no change of concentration of the solution; third: a decrease of concentration with no change in the Δ. These apparent anomalies can be satisfactorily explained by a consideration of the large divergence of the Δ of the two curves in figure 3. It is apparent that as the equilibrium is approached, more and more of substances other than chlorides diffuse into the intestinal contents. Hence, inasmuch as there is an attempt at total osmotic equilibrium as well as partial pressure equilibrium, the concentration of chlorides in the intestine may remain higher than the blood level until replaced by substances but slowly diffusing from the blood. In the cases where the content of the chlorides decreases without a decrease in Δ, other osmotic pressure producing substances have replaced the chlorides.

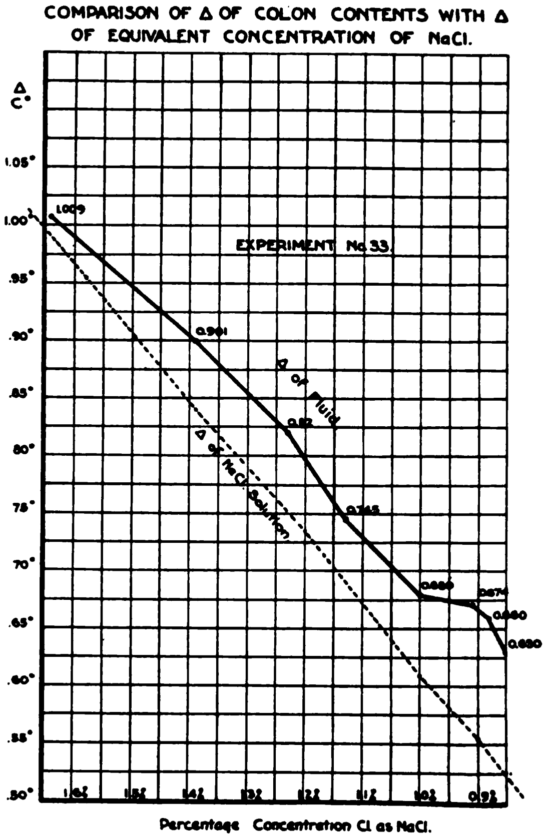

Fig. 3

A protocol given by Diena (5) confirms this point. When he introduces an isotonic solution of sodium chloride into the colon and determines the Δ of the solution recovered, there is little change in the Δ in spite of the fact that there is a considerable decrease in concentration of gut chlorides (from 0.92 to 0.67 per cent). Diena does not comment upon this point.

The observations above may also furnish an analogy and explanation of the findings of Höber that isotonic solutions of sodium chloride occasionally show an increased osmotic pressure during absorption.

CONCLUSIONS

1. Solutions of sodium chloride above or below blood level come into a chloride partial pressure equilibrium with the blood.
2. Increase of blood chlorides affects this equilibrium.
3. There is an attempt at total osmotic pressure equilibrium between the colon contents and the blood.
4. Evidence is at hand which indicates that other blood constituents diffuse into sodium chloride solutions in the colon. This is more apparent as the concentration of the solution approaches the level of the blood chlorides.

BIBLIOGRAPHY

(1) GUMILEWSKY: Pflüger's Arch., 1886, xxxix, 556.
(2) HEIDENHAIN: Pflüger's Arch., 1894, lvi, 579.
(3) WAYMOUTH-REID: Journ. Physiol., 1902, xxviii, 241.
(4) HÖBER: Pflüger's Arch., 1899, lxxiv, 225.
(5) DIENA: Arch. Sci. Mediche, 1911, xxxv, 63.
(6) HAMBURGER: Arch. f. Anat. u. Physiol., 1895, 281.
(7) LEATHES AND STARLING: Journ. Physiol., 1895, xviii, 106.
(8) GOLDSCHMIDT AND DAYTON: This Journal, 1919, xlviii, 49.

STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

IV. The Colon. The Behavior of Sodium and Magnesium Sulphate Solutions

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In previous papers of this series our investigations have been concerned with the behavior of solutions of sodium chloride of various concentrations. It is evident that the intestinal wall behaves toward solutions of sodium chloride like a permeable membrane. A few of the discrepancies, on the basis of our present knowledge, have been pointed out. A priori, in accordance with the conception of the action of sodium sulphate and magnesium sulphate in producing catharsis, we should expect that the behavior of these salts in the colon would present a contrast to the changes occurring with solutions of sodium chloride. In this paper will be presented the changes occurring in the solvent and solute of solutions of sodium and magnesium sulphate.

There is a paucity of literature on this point, from the quantitative point of view. Some conception of the changes occurring in solutions of sodium sulphate and magnesium sulphate in the small and large intestine may be obtained from the classic work of Mathew Hay. The points bearing upon the subject of this paper will be presented briefly. Hay (1) finds that there is a greater flow of fluid into the large intestine, with a given quantity of sodium sulphate, than into the small intestine, with the same area of intestinal surface exposed to the solution. The author suggests two reasons for this difference. First: the mucous secretion into the large intestine inhibits absorption, and second: from the nature of the mucous membrane of the large intestine absorption proceeds with greater slowness than in the small intestine. If the solution of sodium sulphate is sufficiently strong (20 per cent), Hay finds that the amount of fluid withdrawn from the body may lead to a blood concentration, as indicated by an increased number of red cells.

Furthermore, the work of this author indicates most conclusively that, from the small intestine, at least, considerable amount of sodium sulphate is absorbed into the blood. Nearly half of the amount introduced may disappear in the first hour. This factor is of such importance that, if the dose administered does not exceed what can be absorbed during the rapid stage of absorption, there is a failure of catharsis. The amount of sulphate absorbed is also believed to be responsible for a diuresis following the administration of sodium sulphate.

Finally, this investigator finds an "accumulation" of sulphates in the colon, after administration of sodium sulphate. This he ascribes to a combination of two factors, a slower absorption of the salt from the large intestine, which "depends on a difference in the structure and function of the large intestine as compared with the small intestine," and a passage of salt from the blood into the colon contents.

In regard to magnesium sulphate, Hay (2) believes that the salt is split in the intestine, and sulphate passes more easily than the magnesium. It must be added that Hay is not unaware of the relatively low diffusibility of these salts as a factor in their cathartic action.

Kovesi (3) investigated the absorption of sodium sulphate from the small intestine of rabbits, 120 to 150 cm. from the pylorus, and found considerable disappearance of the sulphate. From hypotonic solutions, fluid is absorbed and there is an increased Δ to the level of the blood. With hypertonic solutions there is an increased volume and a decrease in the Δ. Isotonic solutions show little or no change in volume or Δ. The sulphate is absorbed in rather large amounts, according to the author's figures, the absorption increasing with the concentration of the salt introduced. The length of the experiments varies from 1 to 2 hours.

Gumilewski (4) observed, in the small intestine, that sodium sulphate delayed the fluid absorption and that the sulphate absorption increased with increasing concentration. He concluded that the chemical nature of the salts play a part in their absorption.

Heidenhain (5) performed a few experiments with magnesium sulphate in the small intestine, and finds that water is absorbed more slowly than from a solution of sodium chloride of greater osmotic pressure. He concludes that the sodium sulphate lessens the "physiological activity" of the intestinal cells; hence, the factors involved are controlled more by their osmotic pressures than is the case with solutions of sodium chloride.

Wallace and Cushny (6) compared the fluid absorption from a large number of salt solutions with that of a 1 per cent sodium chloride solution. They find that in the list of the cathartic salts the anion is the determining factor in the absorption. Sulphates, standing between the nitrates and chlorides, retard absorption more than chlorides and are identical with the phosphates. These authors find that solutions of all concentrations tend to become isotonic with the blood. Of the cations, they state that those of the alkaline earths are very slowly taken up by the intestinal epithelium. In this investigation the authors measure only fluid absorption and give no data on the fate of the salt introduced, but assume that compared to sodium chloride relatively little is absorbed.

From the pleural cavity, Leathes and Starling (7) report absorption of sodium and magnesium sulphates.

METHODS

In the experiments to be reported below, the same technique was employed as in our previous investigations. The sodium sulphate used for making solutions was in most cases the anhydrous salt. Glauber's salt was found to give similar results. Sulphates were determined gravimetrically, weighed as barium sulphate. The magnesium was determined by the McCrudden (8) method. At the conclusion of the experiment the colon was washed repeatedly with distilled water, the total volume of the washings was about one liter. In computing the amount of sulphate unaccounted for, the sulphates of the washings and samples are deducted from the total amount introduced initially.

EXPERIMENTAL

The experimental results are appended in tables 1 and 2. Considering first the volume changes of the fluid in the colon, it will be noted that with a hypertonic solution of sodium sulphate (8.15 per cent, exper. 21) there is an increase in volume, which is considerable during the first few hours, finally (after 10 hours) remaining stationary within the range of the error of delivery. In experiment 44, where a strongly hypotonic solution is introduced, there is a very rapid absorption of fluid which continues for six hours.

In experiments 46 and 47, where solutions of 2.01 per cent sodium sulphate are used, there is no considerable volume variation in either case; with the exception of the first period of experiment 46, most of the changes are not considerably beyond the experimental error of delivery of the fluid.

The volume changes in the magnesium sulphate experiment cannot be discussed in connection with this salt alone, inasmuch as, for other experimental purposes, sodium chloride was present in the initial solutions. However, in the last period we know from analysis that sodium chloride was present in but very small amount (0.06 per cent

TABLE 1

The behavior of Na_2SO_4 *in the colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME − LOSS + GAIN	Na_2SO_4 INTRODUCED	Na_2SO_4 RECOVERED	Na_2SO_4 INTRODUCED	Na_2SO_4 RECOVERED, FINAL SOLUTION, SAMPLES, WASHINGS	DEFICIT Na_2SO_4	Δ BLOOD	Δ CONTENTS
	mins.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*grams*	*grams*	*grams*		
21	60	25	57	+32	6.64	3.25					
	60	55	72	+17	3.25	2.46					
	60	69	80	+11	2.46	—					
	60	80	87	+ 7	—	2.05					
	60	82	85	+ 3	2.05	—					
	60	85	86	+ 1	—	1.94					
	60	81	75	− 6	1.94	—					
	60	75	77	+ 2	—	1.91					
	60	72	71	− 1	1.91	—					
	60	71	70	− 1	—	1.95	2.445	2.050	0.395		
44	60	250	155	−95	0.50	—				0.582	
	60	155	95	−60							
	60	95	82	−13							
	60	82	48	−34							
	60	48	34	−14							
	60	34	30	− 4		1.65	1.250	1.044	0.206	0.577	0.558
46	60	150	138	−12	2.01	—				0.610	
	60	138	141	+ 3							
	60	141	141	0							
	60	141	139	− 2							
	60	139	140	+ 1							
	60	140	144	+ 4		1.85	3.015	2.778	0.237		0.614
47	60	100	98	− 2	2.01					0.580	
	60	98	99	+ 1							
	60	99	100	+ 1							
	60	100	98	− 2							
	60	98	100	+ 2							
	60	100	100	0		1.56	2.012	1.674	0.338	0.580	0.578

or 0.071 gram). Hence, we may say that the volume increases to a constant level, due to the osmotic pressure of the magnesium sulphate.

The changes in concentration of sodium sulphate in experiments 21 and 44 parallel the changes in volume. In the former (hypertonic solution) the concentration decreases rapidly and becomes fixed at about the same time as the volume. In the latter (hypotonic solution) the concentration is increased during the period of the experiment.

In experiments 46 and 47 there is the anomaly of little fluid change, yet in the latter there is an undoubted diminution of the concentration.

With magnesium sulphate, in experiment 68, on introduction of a hypertonic solution there is a decrease in concentration during the course of the experiment.

TABLE 2

The behavior of $MgSO_4$ *in the colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME, − LOSS, + GAIN	$MgSO_4$ INTRODUCED	$MgSO_4$ RECOVERED	$MgSO_4$ INTRODUCED	$MgSO_4$ RECOVERED	DEFICIT $MgSO_4$	Mg INTRODUCED	Mg RECOVERED	Δ BLOOD	Δ CONTENTS
	mins.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*grams*	*grams*	*grams*	*grams*	*grams*		
68	60	100	113	+13	3.56								
	60	113	125	+12									
	60	125	110	−15									
	60	110	118	+ 8									
	60	118	118	0									
	60	118	118	0									
	60	118	118	0		2.80	3.572	3.468	0.104	0.713	0.714	0.583	0.610

Inasmuch as, in this work, our primary interest centered around the differences in the initial and final solutions, the changes in concentration and osmotic pressure of the solutions were not determined from hour to hour.

The final Δ of the solutions is determined in experiments 44, 46, 47 and 68. In every case it closely approximates the blood Δ, although it will be noted in the first three experiments that the concentration of sulphate in the final solution varies. In experiment 46 a chloride estimation in the final solution shows a concentration of 0.01 per cent or a total of 14 mgm. Also in experiment 68 the chloride concentration in the final solution is 0.06 per cent a total of 70 mgm. The final Δ, therefore, is not, in any considerable part, due to chlorides as we shall show in a following paper.

The amount of sodium sulphate or magnesium sulphate unaccounted for at the end of the experiment is computed. For the sodium sulphate this varies from 200 to 400 mgm. in a period of six to ten hours. In experiment 21, 2.445 grams are introduced with a deficit of 395 mgm. in ten hours.

In experiment 46, the initial solution contained 3.015 grams, and 237 mgm. remained unaccounted for after six hours. In experiment 44, when but 1.25 grams are present, there is a deficit of 206 mgm. in six hours.

In the above calculations the sulphate determined is computed as sodium sulphate for convenience of comparison, but does not imply that all the sulphate was present in the colon as sodium sulphate.

Experiment 68 shows but a slight loss, 104 mgm. of magnesium sulphate (equivalent to 83 mgm. of sulphate). Magnesium was also determined and recovered quantitatively. The grade of sulphate absorption from magnesium sulphate is therefore very low, these figures indicating that it is below the sulphate absorption from solutions of sodium sulphate. None of the magnesium, from this single experiment, appears to be absorbed through the colon.

DISCUSSION

The experiments presented show that hypertonic solutions of sodium or magnesium sulphate gain in volume in the colon of the dog. The concentration decreases and the Δ approaches that of the blood. Hypotonic solutions of sodium sulphate decrease in volume, the concentration rises and the Δ approaches blood level. Solutions of a concentration of sodium sulphate about iso-osmotic with the blood, exhibit little change in volume and approach the Δ of the blood. In this we are in accord with the observations of Kovesi (3), and also those of Wallace and Cushny (6), who showed a similar behavior in the small intestine. The difference of the final concentration of sodium sulphate in the separate experiments is yet to be accounted for. We have shown that chlorides are not present, hence the presence of sodium chloride cannot be the explanation.

The concentration of sodium sulphate equi-osmotic with a 0.95 per cent sodium chloride solution is 1.87 per cent, calculated from the figures of Landolt-Börnstein. For magnesium sulphate it is 2.80 per cent. It will be noted that the final concentrations, in the solutions in the table above, closely approximate these figures.

Only a very small amount of sulphate is absorbed from sodium sulphate solutions in the colon. The work of Mathew Hay (1), (2) indicates that a large amount of sulphate is absorbed from solutions of sodium sulphate in the intestine, for the most part, while the solution is still in the small intestine. This author believes that there is an accumulation of the salt in the large intestine, due to a slow rate of absorption in this region, and to a passage into the large intestine of sulphate from the blood, since the blood is high in content of this salt from absorption from the small intestine. Our results are in complete harmony with the experimental findings of Hay although, on the basis of our studies, we wish to present a somewhat different interpretation of these results. We would suggest that the accumulation of salt in the colon in Hay's experiments is due entirely to sodium sulphate entering from the small intestine, and that this accumulation is only dependent upon the very slight permeability of the colon to the salt. The supposition of passage of sulphate from the blood into the colon, an unlikely phenomenon, is therefore unnecessary. That the volume of the solution is maintained is also due to this fact and not, as Hay (1), (2) suggested, to the mucous content of the colon fluid, for in our experiment with a clean colon this factor was not observed.

On this basis we wish to suggest, with added proof to that already offered by Hay, that this factor of exceedingly slight disappearance of sodium sulphate points to the colon as playing a very important rôle in catharsis. Providing the cathartic salt reaches the colon in sufficient amount, so that by osmotic interchange between the blood and colon enough fluid is introduced to increase the bulk and fluidity of the colon contents, catharsis will be assured. This might occur independent of the volume of fluid reaching the colon from the small intestine. The determining factor will be the amount of the salt which reaches the large intestine, and inasmuch as little is absorbed, this will be cumulative. Furthermore, once enough of an isotonic or hypertonic solution has reached the large intestine to increase the bulk and fluidity of the colon contents, catharsis is assured, for we have shown that but a small quantity of the salt is absorbed in ten hours.

The very small amount of sulphate which remains unaccounted for in our experiments bears no constant relationship to the amount introduced, in fact the figures are of the same order, independent of time of sojourn or concentration of the initial solution. This leads us to believe that we are not dealing with an absorption phenomenon. The other possibility which occurs to us is the adsorption of a rather definite amount of sodium sulphate by the intestinal wall, which may be proportional to the final rather than the initial concentration of sodium sulphate. Hofmeister (9) has shown that colloid substances (agar-agar and gelatine) as well as dried tissue, may take up fluid and salt of solutions in which they are soaked. This varies with different salts. At the end of our experiments the intestine was washed with distilled water, yet there is still the possibility that any sulphate so held by the tissue would escape the washing process.

The very low permeability of the large intestine for magnesium is in accord with the findings of Hay (1), (2) on the elimination of magnesium sulphate. Hay suggests that the magnesium remains in the intestine and that sulphate is absorbed and probably excreted as a sodium compound. It also agrees with the findings of Wallace and Cushny (6) who report "very slow" absorption of the cations of the alkaline earths.

Hofmeister (10) suggests that the cathartic action of magnesium sulphate is due to the formation of magnesium carbonate and sodium sulphate in the intestine. Such an assumption is not necessary on the basis of our work.

CONCLUSIONS

1. The colon behaves toward solutions of sodium sulphate essentially like a semi-permeable membrane. Water is absorbed from hypotonic solutions, and the Δ increases to blood level. With hypertonic solutions the volume increases, the concentration decreases and the Δ approaches blood level. Solutions of concentration nearly iso-osmotic with the blood show little change in volume and the Δ closely approximates the Δ of the blood. Hence, there is free passage of water with practically no diffusion of sulphate.
2. The deficit of sodium sulphate is very small and bears no constant relationship to the total amount of sodium sulphate introduced, nor is it dependent upon the length of stay in the colon, and therefore seems to be due to adsorption rather than to a diffusion.
3. Magnesium sulphate shows even less absorption from the colon than sodium sulphate.
4. The failure of absorption of these salts from the colon emphasizes the specific importance of the large intestine in saline catharsis.

BIBLIOGRAPHY

(1) Hay: Journ. Physiol. and Anat., 1882, xvi, 243, 391, 568.

(2) Hay: Journ. Physiol. and Anat., 1883, xvii, 62, 222, 405; Brit. Med. Journ., 1882, 1204.

(3) Kovesi: Centralbl. f. Physiol., 1897, xi, 553.

(4) Gumilewski: Pflüger's Arch., 1886, xxxix, 556.

(5) Heidenhain: Pflüger's Arch., 1894, lvi, 579.

(6) Wallace and Cushny: This Journal, 1898, i, 411.

(7) Leathes and Starling: Journ. Physiol., 1895, xviii, 106.

(8) McCrudden: Journ. Biol. Chem., 1909, vii, 83.

(9) Hofmeister: Arch. f. Exper. Path. u. Pharm., 1890, xxvii, 395; 1891, xxviii, 210.

(10) Hofmeister: Arch. f. Exper. Path. u. Pharm., 1887, xxiv. 247.

STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

V. THE COLON. THE EFFECT OF SODIUM SULPHATE UPON THE ABSORPTION OF SODIUM CHLORIDE WHEN THE SALTS ARE INTRODUCED SIMULTANEOUSLY INTO THE INTESTINE

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In the preceding paper of this series it has been shown that the colon acts toward solutions of sodium sulphate and magnesium sulphate essentially like a semi-permeable membrane. To sodium chloride solutions, on the other hand, the colon resembles in many respects a permeable membrane. The cause of this difference in behavior to the same surface is of extreme interest. Is it due to the effect of the salt, per se, upon the cells or walls of the colon whereby the permeability of the colon is altered? This is indeed one possibility which it is our purpose to put to a test in the following experiments.

Starling (1), discussing intestinal absorption, says:

> If, on the other hand, salts, such as sodium sulphate, which pass with extreme difficulty through the cell lining are present, the osmotic pressure, due to the dissolved salts, continues as a force opposing the absorptive activity of the cells.

Heidenhain (2) finds that there is less absorption of water from magnesium sulphate solutions, in the small intestine, than from sodium chloride solutions of equal or even greater osmotic pressure. The author points out that, if water absorption from the intestine were dependent upon osmotic pressure factors, equi-osmotic solutions should have the same water absorption. Hence, Heidenhain ascribes this action of magnesium sulphate to an influence upon the "physiological activity" of the intestinal cells. In order to increase the osmotic pressure of solutions introduced into the small intestine, without changing the amount and concentration of sodium chloride, this investigator adds "an indifferent neutral salt, sodium sulphate." He states that both the absorption of fluid and of chloride may diminish. The author adds that the action is purely physical and disappears if the intestine is well washed.

Wallace and Cushny (3) discuss the probable cause of the non-absorption of the cathartic salts, and suggest as a possible explanation the precipitation of calcium in the intestinal wall by these salts. They give as analogies the effect of the precipitation of calcium in coagulation of blood and of milk. These investigators state that while sodium sulphate does not, as a general rule, impair the absorption of intestinal loops permanently, they infer that there is an injurious effect.

Hay (4) discusses the effect of solutions of sodium sulphate upon the absorptive power of the intestine. In order to test this point he injects strychnine into sodium sulphate solutions, in ligatured loops of the small intestine of cats. Hay concludes that the intestine retains its powers of absorption even when a 20 per cent solution of a purgative salt has been used, and also that the absorptive power of the intestine is less injured by a weak solution than by a strong solution of the salt. He further believes to have demonstrated that strong solutions of sodium sulphate retard absorption of weak solutions subsequently introduced.

Waymouth-Reid (5) finds that when sodium chloride is added to glucose solution in the small intestine, the uptake of glucose is favored at the same time that water is retarded by the salt. He concludes that this is evidence that "chemical excitation" may play a part in intestinal absorption.

Hamburger (6), in a study of the changes in volume of isolated epithelial cells of the small intestine, subjects them to sodium chloride and magnesium sulphate solutions. Magnesium sulphate in increasing concentration swells the cells. When the cells are merely soaked in magnesium sulphate, and then subjected to increasing concentrations of sodium chloride, Hamburger finds that there is a greater power of diminishing the volume of the cells, than when the cells are merely subjected to. sodium chloride alone in increasing concentration. He believes that magnesium sulphate alters the state of permeability of the cells.

METHODS

The experimental procedures and methods of analysis employed in this investigation are similar to those in previous papers of the series. In comparing the total amount of chlorides absorbed when increasing amounts of sodium sulphate are added to the solution, the concentration of chlorides, volume of solution introduced and length of stay in the intestine, have been kept constant. Hence, the only variables are the sodium sulphate and osmotic pressure of the solution. A low concentration of sodium chloride has been selected for this purpose in order to give as large a range of concentration as possible below the osmotic pressure of the blood, and thus avoid the interfering factor of fluid inflow from the blood. The sodium sulphate was the anhydrous salt.

EXPERIMENTAL

The data in table 1, above, demonstrate that when a solution containing sodium sulphate and sodium chloride is introduced into the colon, the chlorides are absorbed to completion, while the sulphate is responsible for nearly the total osmotic pressure equilibrium between the gut contents and the blood. The initial concentration of sodium sulphate is that of a solution slightly hypertonic with the blood. In both experiments the final concentration of sodium sulphate approaches the concentration of a solution equi-osmotic with the blood. The final Δ of the gut contents also shows little variation from that of the blood. Since examples for analysis were removed at the end of each period we

TABLE 1

The effect of Na_2SO_4 *upon diffusion of* NaCl *through colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME: − LOSS, + GAIN	Na_2SO_4 INTRODUCED	Na_2SO_4 RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	Δ CONTENTS	Δ BLOOD	
	min.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*			
34	60	96	94	− 2	2.00		0.92	0.49			
	60	90	77	−13			0.49	0.30			Contents returned
	60	73	61	−12			0.30	0.15			Contents returned
	60	51				1.72	0.15	0.04	0.585	0.590	Contents returned
48	60	80	90	+10	2.00		0.60		0.945	0.596	Contents returned
	60	90	94	+ 4							Contents returned
	60	94	93	− 1							Contents returned
	60	93	87	− 6				0.06			Contents returned
	60	77	74	− 3		1.83	0.06	0.01	0.620	0.590	

cannot get a proper picture of the changes in the volume of fluid, but it is evident that they are slight except in the first periods.

Of greater interest is the change in the sodium chloride content. In experiment 34, the initial solution introduced contained 0.92 per cent sodium chloride, there is a rapid and progressive decrease in concentration until the final solution contains but 0.04 per cent. A solution of 0.6 per cent (480 mgm.) was introduced in experiment 48 and the final solution contained but 0.01 per cent (7 mgm.). During the last hour of this experiment the solution was repeatedly removed and returned so that absorption was facilitated by mixing.

TABLE 2

Effect of $MgSO_4$ *upon diffusion of* NaCl *through colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME	$MgSO_4$ INTRODUCED	$MgSO_4$ RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	Δ CONTENT	Δ BLOOD	REMARKS
	min.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*			
50	60	90	81	− 9	1.00		0.81	0.67			Solution of $MgSO_4$ undergoing concentration with disappearance of NaCl
	60	71	58	−13			0.67	0.54			
	60	48	29	−19		1.57	0.54	0.39			
68	120	85	125	+40	3.56		0.82				Solution of $MgSO_4$ undergoing dilution with disappearance of NaCl
	120	125	118	− 7							
	180	118	118	0		2.80		0.06	0.610	0.583	

Table 2 shows two experiments which demonstrate the behavior of sodium chloride in the presence of magnesium sulphate in the large intestine. These data present precisely the same features as are shown in table 1 with sodium sulphate. With a concentration of magnesium sulphate hypertonic with the blood (exper. 68,) the chlorides are rapidly absorbed and the amount present rapidly approaches a zero value (0.06 per cent or 71 mgm.). The concentration of magnesium sulphate is nearly that of a solution equi-osmotic with the blood. The Δ of the final solution closely approximates that of the blood.

With a concentration of magnesium sulphate hypotonic with the blood (exper. 50,) both fluid and chlorides are rapidly absorbed. There is every indication that if the experiment were continued for a sufficient period of time, the chlorides would diminish to a very low figure, and the magnesium sulphate reach a concentration of a solution equi-osmotic with the blood.

It has been shown in previous work that chlorides pass into distilled water in the large intestine (7). In table 3 have been compiled a few typical protocols which show the degree to which this phenomenon occurs when the fluid in the colon is sodium sulphate. These data demonstrate that the total amount of chlorides passing into dilute solutions of sodium sulphate is less than that passing into water (exper. 40) and that the percentage concentration is also lower. Furthermore, it will be noted that the order of magnitude of chloride concentration and total amount is less in solutions of sodium sulphate as the concentration of the latter increases (exper. 41, 33 and 34.)

TABLE 3

The passage of chlorides into solutions of sodium sulphate in colon

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	Na_2SO_4 INTRODUCED	NaCl RECOVERED	AMOUNT NaCl RECOVERED
	min.	*cc.*	*cc.*	*per cent*	*per cent*	*grams*
40	30	100	90	0	0.09	0.081
	30	100	88	0	0.08	0.070
	30	100	87	0.15	0.05	0.043
	30	100	87	0.15	0.03	0.026
	30	100	83	0.15	0.04	0.033
	30	95	82	0.30	0.03	0.025
41	30	140	81	0.50	0.01	0.011
33	120	195	126	1.00	0.02	0.24
34	45	100	95	2.00	0.02	0.16

Up to this point it has been shown that sodium chloride in the presence of either sodium or magnesium sulphate in the large intestine is absorbed and after a time approaches a zero value. Further experiments show that sodium sulphate influences the speed of absorption of sodium chloride. The results are given in table 4.

In the experiment presented (79), increasing concentrations (0.1 to 2.0 per cent) of sodium sulphate, containing a constant concentration of sodium chloride, are introduced into the intestine. Since the volume of the solutions introduced is constant, the total amount of sodium chloride in each solution is, therefore, the same. The time which the solution is allowed to remain in the intestine is also the same in all periods. The range of osmotic pressure in the sodium sulphate solutions is from a strongly hypotonic to hypertonic, as compared to the blood. Experimental periods (sodium sulphate) are always alternated with control periods (sodium chloride alone). After each period in which sodium sulphate has been used, the colon is washed with saline (0.3 per cent), in order to remove as much as possible of the sodium sulphate.

TABLE 4

Effect of sodium sulphate upon the speed of diffusion of NaCl *in the colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	VOLUME CHANGE: + GAIN, − LOSS	Na_2SO_4 INTRODUCED	NaCl INTRODUCED	NaCl RECOVERED	CHANGE NaCl: + GAIN, − LOSS	CHANGE NaCl: − LOSS	Δ SOLUTION INTRODUCED	Δ CONTENTS RECOVERED	CONC. NaCl SOLUTION ABSORBED
Experiment 79	mins.	cc.	cc.	cc.	per cent	per cent	per cent	per cent	mgm.			per cent
Period 1	30	100	76	−24	0	0.3	0.36	+0.06	−26	0.210	0 275	0.11
	30	100	75	−25	0	0 3	0.35	+0.05	−37	0.205	0.265	0.15
	30	100	75.5	−24.5	0	0.3	0.35	+0.06	−36	0.200	0.256	0:15
	30	100	71 5	−28.5	0.10	0.3	0.33	+0.03	−64	0.228	0.297	0.23
Period 2	The gut washed with 300 cc. of 0.3 per cent NaCl solution											
	30	100	71.5	−28.5	0	0.3	0.34	+0.04	−57	0.206	0.252	0.20
	30	100	76	−24	0.50	0.29	0.28	−0.01	−77	0.353	0.404	0.32
Period 3	Gut washed with 400 cc. of 0.3 per cent NaCl solution											
	30	100	70	−30	0	0 3	0.85	+0.05	−55	0.210	0.259	0.18
	30	100	91	− 9	1.0	0.3	0.24	−0.06	−82	0 498	0.513	0.91
Period 4	Gut washed with 400 cc. 0.3 per cent NaCl solution											
	30	100	72	−28	0	0.3	0.35	+0.05	−48	0.202	0.256	0.17
	30	100	100	0	1 5	0.3	0.22	−0.08	−80	0.646	0.611	
Period 5	Gut washed with 400 cc. of 0.3 per cent NaCl solution											
	30	100	71	−29	0	0.3	0.36	+0.06	−44	0.195	0.260	0.15
	30	100	107	+ 7	2.0	0.29	0.21	−0.08	−65	0.775	0.709	
Period 6	Gut washed with 400 cc. of 0.3 per cent NaCl solution											
	30	100	72.5	−27.5	0	0.30	0.36	+0.06	−39	0.195	0.258	0.14
	30	100	75	−25	0	0.30	0.33	+0.03	−52	0.198	0.236	0.21

It will be noted that, with increasing concentration of sodium sulphate, the total amount of chlorides absorbed, in every case, exceeds that of the corresponding control period. This is shown graphically in figure 1. This is true regardless of whether the experimental solutions are hypotonic (periods 1, 2 and 3) or hypertonic (periods 4 and 5.) The volume of fluid absorbed, on the other hand, is progressively decreased, so that the calculated concentration of the chloride solution absorbed shows increasing values in the experimental periods. It should be observed that whereas in the control periods the final concentration of sodium chloride is always above that of the initial solution, the reverse is the case when sodium sulphate is added in the experimental periods. As will be seen from the table, this diminution in the concentration of chlorides increases as the concentration of sodium sulphate is raised in the experimental solution.

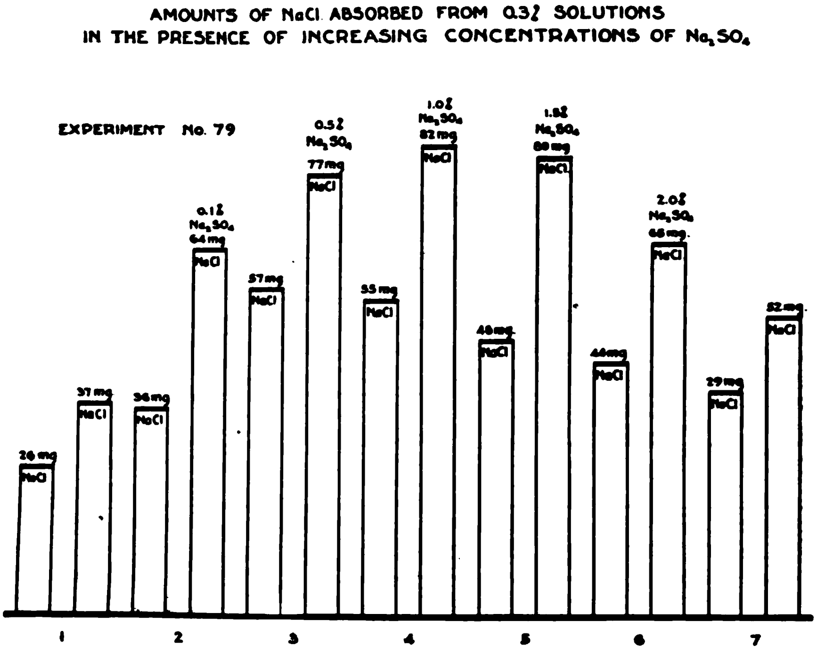

Fig. 1

Another point is evident when one compares the amount of chlorides absorbed in the different control periods (table 4). The control of period 2, which follows the first period in which sodium sulphate is used, shows an undoubted increase of chloride absorption over the preceding control periods. This higher level of chloride absorption

TABLE 5

Effect of sodium sulphate upon colon wall

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME; − LOSS, + GAIN	Na_2SO_4 INTRODUCED	NaCl INTRODUCED	NaCl RECOVERED	CHANGE NaCl IN CONTENTS; − LOSS	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	REMARKS
	mins.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*mgm.*			
Experiment 80											
Period 1..	30	100	77	−23	0	0.31	0.33	−56	0.216	0.250	
	30	100	78	−22	0	0.31	0.33	−53	0.214	0.254	
	30	100	75.5	−24.5	0	0.31	0.33	−61	0.212	0.247	Average absorption = 57 mgm.
Period 2..	2 per cent Na_2SO_4 introduced into gut for 30 minutes and washed out with 0.3 per cent NaCl until washings were free from SO_4										
Period 3..	30	100	159	−41	0	0.31	0.38	−86	0.208	0.290	
	30	100	67.5	−32.5	0	0.31	0.35	−74	0.208	0.268	
	30	100	67	−33	0	0.31	0.35	−75	0.206	0.265	Average absorption = 78 mgm.
Period 4..	2 per cent Na_2SO_4 introduced into gut for 30 minutes and washed out with 0.3 per cent NaCl until washings were free from SO_4										
Period 5..	30	100	67	−33	0	0.31	0.35	−75	0.213	0.270	
	30	100	65	−35	0	0.31	0.35	−82	0 215	0.265	Average absorption = 78 mgm.
Experiment 81											
Period 1....	30	100	62	−38	0	0.31	0.36	−87			Average absorption = 87 mgm.
Period 2..	1 per cent Na_2SO_4 introduced into gut for 30 minutes and washed out with 0.3 per cent NaCl until washings were free from SO_4										
Period 3..	30	100	56	−44	0	0.30	0.37	−93			
	30	100	62	−38	0	0.31	0.37	−81			Average absorption = 87 mgm.
Period 4....	30	100	79	−21	1.0	0.31	0.25	−112			
Period 5....	30	100	62	−38	0	0.31	0.37	−81			
Period 6....	2 per cent Na_2SO_4 introduced into gut for 30 minutes and washed out with 0.3 per cent NaCl until washings were free from SO_4										
Period 7....	30	100	63	−37	0	0.31	0.38	−71			

persists in the remaining control periods of the experiment. Table 5 (above) gives two typical experiments which demonstrate that the effect just described is a constant one after sodium sulphate has been in the colon.

TABLE 6

Effect of sodium sulphate contrasted with mechanical washing and various concentrations of NaCl *and distilled water upon the colon wall*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME: −LOSS +GAIN	NaCl INTRODUCED	NaCl RECOVERED	CHANGE NaCl: −LOSS	REMARKS
Experiment 82	*min.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*mgm.*	
Period 1...	30	100	78	−22	0.31	0.34	−45	
Period 2...	Washed intestine with 950 cc. of 0.31 per cent NaCl solution							
Period 3...	30	100	76	−24	0.31	0.34	−52	
Period 4...	Washed intestine with 500 cc. of 0.31 per cent NaCl solution							
Period 5...	30	100	75.5	−24.5	0.31	0.34	−53	
Period 6...	Allowed 1 per cent NaCl to remain in gut for 30 minutes							
Period 7...	30	100	71.5	−28.5	0.31	0.35	−60	
Period 8...	30	100	91	− 9	0.31	0.26	−83	Solution contained 1 per cent Na_2SO_4. Note increased NaCl loss
Experiment 83								
Period 1...	30	100	80	−20	0.31	0.34	−38	
Period 2...	Washed intestine with 400 cc. of 0.31 per cent NaCl solution							
Period 3...	30	100	77.5	−22.5	0.30	0.33	−44	
Period 4.	Washed intestine with distilled water and allowed it to remain in gut for 30 minutes							
Period 5...	Then washed intestine with 200 cc. of 0.31 per cent NaCl							
Period 6...	30	100	80	−20	0.30	0.32	−44	
Period 7.	Washed intestine with 0.6 per cent NaCl solution and allowed it to remain in gut for 30 minutes. Then washed intestine with 200 cc. of 0.31 per cent NaCl							
Period 8...	30	100	74	−26	0.30	0.33	−56	
Period 9	Allowed 1.98 per cent NaCl to remain in gut 30 minutes. Then washed intestine with 250 cc. of 0.31 per cent NaCl solution							
Period 10..	30	100	73	−27	0.30	0.35	−44	

There is little doubt that in period 3, experiment 80, after a 2.0 per cent sodium sulphate solution is allowed to remain for thirty minutes in the intestine, the chloride absorption from solutions of sodium chloride is of a higher grade than in period 1. However, a repetition of this procedure does not increase this latter grade of chloride absorption, as is indicated in periods 4 and 5. This point is further substantiated in experiment 81. Period 1 represents a period following the presence of sodium sulphate in the intestine for other experimental purposes. It can be seen that the amount of chloride absorbed cannot be increased by subsequent treatment of the colon with sodium sulphate. However, the same percentage concentration of sodium sulphate, introduced simultaneously with the sodium chloride, increases the amount of absorbed chlorides greatly in period 4.

It seemed desirable to test whether the effect just described may be due to the mechanical process of repeatedly washing the intestine with salt solution, giving a cleaner absorptive surface. In addition, it is of interest to know whether the effect, whatever its cause, is peculiar to sodium sulphate, or whether treatment of the intestine with other solutions than those employed would produce the same result. The results are uniformly negative and are compiled in table 6.

In experiment 82 mechanical washing shows no effect upon the grade of chloride absorption. The amounts of sodium chloride absorbed do not show any marked variation from period to period. In period 8, however, the addition of sodium sulphate gives the characteristic increase of chloride absorption.

In experiment 83 it is shown that neither distilled water, 0.6 per cent, nor 1.98 per cent sodium chloride solution have an effect upon the amount of chlorides subsequently absorbed from solutions of 0.3 per cent of this salt.

These features are discussed below.

DISCUSSION

The data presented above show conclusively that when sodium chloride and either sodium sulphate or magnesium sulphate are present in the colon, the amount of chlorides decreases. The percentage concentration of these sodium chloride solutions progressively decreases, which is a reversal of the normal behavior of solutions of sodium chloride alone. The concentration of sodium or magnesium sulphate approaches that of a solution equi-osmotic with the blood.

The Δ of the solution also approximates that of the blood, thus, the sodium or magnesium sulphate makes up, as far as we may judge at present, the total osmotic pressure equilibrium between the colon contents and the blood.

It has been further shown that smaller quantities of sodium chloride pass into weak solutions of sodium sulphate than into distilled water in the colon. This effect is more exaggerated with increasing concentrations of sodium sulphate. The presence of sodium sulphate in sodium chloride solutions increases the speed of absorption of chlorides. In the course of the experiments certain observations were made which may throw some light upon the cause of this speeding effect. It is noticed that after sulphates have been present in the intestine, the subsequent control periods, with solutions of pure sodium chloride, show an increase in the chloride absorption. This increased chloride absorption rises to a maximum usually after the first introduction of the sodium sulphate solution, and is maintained at almost the same level in all of the following control periods. It is difficult to ascribe this effect to only sulphates retained in the intestine, inasmuch as it is constant, regardless of the concentration of sulphate introduced in the experimental periods. Another factor which we have thought might in part account for this "lag" is the mechanical effect of washing the intestine, but our experiments on this point give negative results.

The possibility arises that the speeding effect is due to an action of the sodium sulphate upon the permeability of the intestinal epithelium. We have tried in other ways to influence the permeability of the membrane, by introduction of hypertonic solutions of sodium chloride and by distilled water, with negative results. Hence, at this stage of our investigations this problem is unsettled.

We wish to suggest, as another possible explanation, that the total osmotic pressure of the solution is an important factor in determining the rate and direction of the passage of diffusible substances through the intestine, whether or not this osmotic pressure is due to diffusible or non-diffusible substances.

The inability of distilled water and high concentrations of sodium chloride (1.98 per cent) to affect the subsequent absorptive capacity of the intestinal wall proves that the intestinal epithelium is not injured. This is of interest in connection with the observations of Waymouth-Reid (8) that, in portions of the small intestine, the epithelium is very susceptible to strength of solution employed and especially to distilled water. He reports there mere washing of a loop of the small intestine with distilled water shows less absorption from the animal's own serum than a control washed with 0.9 per cent sodium chloride. These observations are not in accord with our experiments.

Within the range of concentration of sodium sulphate, used in our experiments, there is absolutely no evidence that the presence of sodium sulphate injures the intestinal epithelium as suggested by Hay (4) and Wallace and Cushny (3), and thereby impedes absorption of sodium chloride from solutions subsequently introduced.

Our results are also contrary to those of Heidenhain (2), using the small intestine, that there is a retardation of salt absorption when sodium sulphate is added to sodium chloride. In regard to water absorption we are in agreement.

The cause of the failure of passage of sodium sulphate through the colon wall is of utmost interest.

Hamburger (6), from his experiments upon isolated epithelial cells, believes that magnesium sulphate alters the state of permeability of the cells. From our experiments we must conclude that, if this be the case, the effect must be an increased permeability, at least for sodium chloride.

The suggestion of Wallace and Cushny (3) that the probable explanation of the cause of the low absorption of the cathartic salts is the precipitation of calcium in the intestinal wall, is of interest in this connection. If these authors imply that this is the factor influencing the permeability of the epithelium, we cannot agree. For, if this be true, the absorption of sodium chloride as well as sodium sulphate should be impeded. Our experiments show that not only is the absorptive capacity of the intestinal wall for sodium chloride not decreased but, on the contrary, is increased under these conditions.

The effect of sodium sulphate upon sodium chloride is not without analogy in the body. Hamburger (9) finds that on injection of a hypertonic solution of sodium sulphate into the blood of dogs and horses, there is a reduction of the sodium chloride, carbonate and protein content of the serum, although the osmotic pressure of the blood returns to normal very quickly. Hypotonic solutions have the same effect. He believes that this is due to the slowness with which sodium sulphate leaves the blood.

Cushny (10) finds in rabbits that when sodium sulphate and sodium chloride are injected together into the blood stream, the chlorides of the urine rise rapidly not only in absolute amount but also in percentage, followed by a rapid fall. The sulphate excretion, on the other hand, falls more slowly and is present in the urine after the chlorides have disappeared. Sulphates injected alone also give a large excretion of chlorides. The author's explanation of this phenomenon is the difference in the reabsorption of sodium chloride and sodium sulphate in the kidney. This investigator says that the epithelium of the renal tubules resembles that of the intestine histologically and physiologically in its reaction to chlorides, phosphates and sulphates.

Magnus (11), in a study of the diuresis produced by various salts in dogs and rabbits finds that, when sodium sulphate is injected into the blood, chlorides sink as the sulphate content rises in the blood. He believes that chlorides go into the tissues. This investigator does not observe the increased chlorides in the urine.

SUMMARY

1. When sodium sulphate or magnesium sulphate is added to solutions of sodium chloride and introduced into the colon, the concentration of chlorides diminishes rapidly and the amount present approaches a zero point.
2. The concentration of sodium or magnesium sulphate approaches a value of a concentration equi-osmotic with the blood. The final Δ of the solution approximates that of the blood.
3. Less chlorides diffuse into weak solutions of sodium sulphate than into distilled water and this amount decreases with increasing concentration of sodium sulphate.
4. Sodium sulphate speeds the rate of absorption of sodium chloride through the colon wall when the two are simultaneously introduced. This increases with increasing concentration of the sulphate. It occurs in hypotonic as well as in hypertonic solutions.
5. After sodium sulphate has been present in the intestine the subsequent absorption of sodium chloride and water to some extent increases. It is never decreased.
6. Other factors such as mechanical washing, introduction of various strengths of sodium chloride, and notably distilled water, do not affect the subsequent absorption of sodium chloride or fluid in the colon.
7. Analogies of the effect of sodium sulphate upon chlorides, in other parts of the body, are presented from the literature.

BIBLIOGRAPHY

(1) Starling: The fluids of the body, 1909 (The Herter Lectures, New York, 1908).
(2) Heidenhain: Pflüger's Arch., 1894, lvi, 578.
(3) Wallace and Cushny: This Journal, 1898, i, 411.
(4) Hay: Journ. Anat. and Physiol., 1882, xvi, 391.
(5) Waymouth-Reid: Journ. Physiol., 1902, xxxviii, 241.
(6) Hamburger: Osmotischer Druck und Ionenlehre, 1904, iii, 7–21.
(7) Goldschmidt and Dayton: This Journal, 1919, xlviii, 419.
(8) Waymouth-Reid: Journ. Physiol., 1898, xxii, 56.
(9) Hamburger: Zeitschr. f. Biol., 1890, xxvii, 259; Osmotischer Druck und Ionenlehre, ii, 1–20.
(10) Cushny: Journ. Physiol., 1901, xxvii, 429.
(11) Magnus: Arch. f. Exper. Path' u. Pharm., 1900, xliv, 396.

STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

VI. THE COLON. THE INFLUENCE OF CALCIUM SALTS UPON THE ABSORPTION OF SODIUM CHLORIDE IN THE INTESTINE

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It has been demonstrated that sodium sulphate has a speeding effect upon the diffusion of sodium chloride through the colon wall. One possible explanation of this phenomenon is an effect of the sodium sulphate upon the permeability of the intestinal wall. In view of the well-known antagonistic action of calcium upon sodium chloride, supposedly related to permeability changes, we have studied the effects of calcium salts upon the passage of chlorides out of the colon.

The researches of Loeb (1) lead him to believe that the antagonistic effects described by him are dependent upon the salts concerned preventing each other from entering the cells or to a difference in the rate of diffusion of the salt mixture through the membrane, and not an effect upon the protoplasm.

Osterhout (2) is of the same opinion as regards the hindering effects of the salts on each other, but finds that even balanced solutions may enter the cell, if but slowly. He believes that the antagonistic substances affect certain life processes which control permeability. The preservation of normal permeability according to the investigator is the result rather than a cause of antagonism. This author favors the view of a change in properties of the membrane.

Lillie (3) also believes that the "antitoxic" action of calcium salts upon sodium salts is due to a prevention or retardation of the increase in permeability produced by the latter salts alone.

Mathews (4) states that calcium may increase the permeability of the membrane of the fundulus egg for some ions and reduce it for others.

Clowes (5) reports that salts like $CaCl_2$ diminish the permeability of protoplasmic membranes for water, while salts like NaOH and NaCl increase the permeability of the membrane. Although antagonism is due to a balance between all cations and anions involved, it happens that $CaCl_2$ has a dominant cation while NaCl has a dominant anion.

The inhibitory action of calcium on body phenomena has been demonstrated. MacCallum (6) observed that $CaCl_2$ may exert an inhibitory effect upon the diuretic action of certain hemolytic substances and in addition inhibit the hemolysis. Davis (7) has found that $CaCl_2$ intravenously causes urinary secretion to diminish and will antagonize the diuretic action of sodium chloride.

The following data are presented as a preliminary report.

METHODS

The experimental methods were the same as described in the first paper of this series (8). The calcium lactate $Ca(C_3H_5O_3)_2.5\ H_2O$ was accurately weighed. The composition of all the calcium lactate solutions is expressed as grams of calcium lactate per 100 cc. of solution. The calcium chloride was the anhydrous salt, and the concentrations are similarly expressed.

EXPERIMENTAL

The data, compiled in the protocols of table 1 demonstrate the accelerating effect followed by the inhibitory action of increasing concentration of calcium lactate upon the passage of chlorides of sodium chloride from the colon.

In experiments 2 and 3, with a level of sodium chloride of 0.6 per cent, it will be noted that with increasing concentrations of calcium lactate, a point is reached where the total amount of chlorides absorbed is diminished. This is shown in experiment 2, by a slight diminution of chloride absorption in period 5. A more marked effect is shown in period 6, with a higher concentration of calcium lactate. The solutions of period 5 are isotonic and those of period 6 hypertonic with the blood. In experiment 3, the first slight inhibitory action is in period 6. The next two periods, 7 and 8, in which the calcium lactate concentration is progressively increased, show a marked decrease in the chloride absorption. Period 10, which corresponds in concentration of calcium lactate to period 6, exhibits a decrease from the preceding control period (9). All of these solutions are hypertonic with the blood.

TABLE 1

The effect of Ca *upon water and chloride absorption in the colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME FLUID, +GAIN −LOSS	NaCl INTRODUCED	CA LACTATE INTRODUCED	NaCl RECOVERED	CHANGE NaCl, +GAIN −LOSS	CHANGE NaCl −LOSS	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	RATIO Ca: Cl
	min.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*			
Experiment 2												
Period 1...	30	85	60	−25	0.62	0	0.64	+0.02	−143	0.390	0.414	
Period 2...	30	85	60	−25	0.63	0.1	0.63	0	−158	0.423	0.439	1: 21
Period 3...	30	85	65	−22	0.63	0.3	0.60	−0.03	−146	0.466	0.484	1: 7.0
Period 4...	30	85	69	−16	0.63	0.6	0.56	−0.07	−149	0.527	0.530	1: 3.5
Period 5...	30	85	74	−11	0.62	0.9	0.55	−0.07	−120	0.586	0.586	1: 2.6
Period 6...	30	85	81.5	− 3.5	0.61	1.38	0.57	−0.04	− 54	0.674	0.664	1: 1.5
Experiment 3												
Period 1...	30	85	62.5	−22.5	0.63	0	0.69	+0.06	−104			
Period 2...	30	85	69	−16	0.63	0.42	0.62	−0.01	−108			1: 4.9
Period 3...	30	85	70	−15	0.63	0.63	0.58	−0.05	−130			1: 3.3
Period 4...	30	85	71	−14	0.63	0.85	0.57	−0.06	−131			1: 2.4
Period 5...	30	85	76	− 9	0.63	1.06	0 54	−0.09	−125			1: 1.9
Period 6...	30	85	80.5	− 4.5	0.62	1.42	0.51	−0.11	−117			1: 1.4
Period 7...	30	85	89	+ 4	0.62	2.65	0.49	−0.13	− 91	0.802	0.708	1: 0.8
Period 8...	30	85	91	+ 6	0.62	3.19	0.48	−0.14	− 90	0.946	0.851	1: 0.6
Period 9...	30	85	55.5	−29.5	0.63	0	0.70	+0.07	−147	0.421	0.481	
Period 10..	30	85	78.0	− 7	0.63	1.49	0.53	−0.10	−122	0.630	0.666	1: 1.4
Experiment 4												
Period 1	30	85	64	−21	0.35	0	0.41	+0.06	− 35	0.245	0.300	
	30	85	60	−25	0.35	0	0.41	+0.06	− 52	0.239	0.292	
	30	85	49	−36	0.34	0.35	0.36	+0.02	−113	0.306	0.363	1: 3.2
Period 2	30	85	52.5	−32.5	0.34	0	0.40	+0.06	− 79	0.236	0.278	
	30	85	72	−13	0.34	1.06	0.31	−0.03	− 66	0.436	0.452	1: 1
Period 3	30	85	44	−41	0.34	0	0.40	+0.06	−113	0.238	0.286	
	30	85	75	−10	0.34	1.59	0.30	−0.04	− 64	0.511	0.526	1: 0.7
	Washed intestine with 0.34 per cent NaCl solution to remove traces Ca lactate											
Period 4	30	85	40	−45	0.34	0	0.42	+0.08	−121	0.240	0.306	
	30	85	82	− 3	0.34	2.12	0.30	−0.04	− 43	0.626	0.597	1: 0.5
Period 5	30	85	46	−39	0.34	0	0.41	+0.07	−100	0.232	0.289	
	30	85	84	− 1	0.34	2.50	0.30	−0.04	− 37	0.658	0.655	1: 0.45
Period 6	30	85	52	−33	0.34	0	0.41	+0.07	− 76	0.228	0.293	
	30	85	87	+ 2	0.34	3.00	0.30	−0.04	− 28	0.764	0.730	1: 0.37
Period 7	30	85	58	−27	0.34	0	0.41	+0.07	− 51	0.239	0.273	
	30	85	61	−24	0.34	0	0.39	+0.05	− 51	0.228	0.267	

TABLE 1—*Continued*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME FLUID +GAIN −LOSS	NaCl INTRODUCED	CA LACTATE INTRODUCED	NaCl RECOVERED	CHANGE NaCl, +GAIN − LOSS	CHANGE NaCl − LOSS	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	RATIO Ca:Cl
Experiment 5	*min.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*			
Period 1	30	100	68	−32	0.31	0	0.35	+0.04	− 72	0.204	0.269	
	30	96	60	−36	0.31	0	0.35	+0.04	− 88	0.199	0.240	
	30	100	59	−41	0.31	0	0.35	+0.04	−104	0.206	0.242	
	30	100	65	−35	0.31	0	0.35	+0.04	− 83	0.209	0.257	
	30	92	48	−44	0.31	0.1	0.33	+0.02	−127	0.223	0.261	1: 10
Period 2	Washed intestine with 100 cc. 0.3 per cent NaCl solution											
	30	100	61	−39	0.31	0	0.34	+0.03	−103	0.204	0.236	
	30	100	58	−42	0.31	0.3	0.32	+0.01	−124	0.271	0.320	1: 3.5
Period 3	Washed intestine with 150 cc. 0.31 per cent NaCl solution											
	30	100	60	−40	0.30	0	0.36	+0.06	− 84	0.213	0.261	
	30	100	72	−28	0.30	0.6	0.30	0	− 84	0.332	0.372	1: 1.7
Period 4	Washed intestine with 150 cc. 0.31 per cent NaCl solution											
	30	100	60	−40	0.30	0	0.36	+0.06	− 84	0.209	0.257	
	30	100	77	−23	0.30	0.9	0.29	−0.01	− 77	0.376	0.406	1: 1.1
Period 5	Washed intestine with 200 cc. 0.31 per cent NaCl solution											
	30	100	54	−43	0.31	0	0.36	+0.05	−116	0.202	0.264	
	30	100	82	−18	0.30	1.2	0.27	−0.03	− 79	0.420	0.443	1: 0.8
Period 6	Washed intestine with 250 cc. 0.31 per cent NaCl solution											
	30	100	55	−45	0.31	0	0.35	+0.04	−118	0.209	0.251	
	30	100	99	− 1	0.30	2.4	0.25	−0.05	− 53	0.621	0.581	1: 0.4
Period 7	Washed intestine with 300 cc. 0.31 per cent NaCl solution											
	30	100	58	−42	0.30	0	0.35	+0.05	− 97	0.209	0.259	
	Washed intestine with 350 cc. 0.31 per cent NaCl solution											
	30	100	57	−43	0.31	0	0.35	+0.04	−111	0.210	0.279	

In order to afford a greater range of concentrations below isotonicity, the concentration of sodium chloride in the next two experiments is decreased. In addition, each alternate period is made a control, keeping constant the concentration and amount of sodium chloride.

Experiments 4 and 5 give some new features and bring out more clearly what has been indicated in the preceding experiments. In both experiments two effects are observed when calcium lactate, in increasing concentrations, is added to sodium chloride. First: at low concentrations of calcium lactate, the amount of chlorides absorbed is increased. This is evident in period 1 of experiment 4 and periods 1 and 2 of experiment 5. Second: at higher concentrations of calcium lactate there is an opposite effect, namely, a decrease in the amount of chlorides absorbed. In experiment 4, all of the experimental periods, after 1, show this inhibitory effect. It is also demonstrated, in experiment 5, in the experimental periods following no. 3. An intermediate effect is seen in period 3 of experiment 5. With the concentration of calcium lactate used in this solution, the amount of chlorides absorbed does not differ from that of the control period where sodium chloride alone is subjected to absorption. All of these changes may occur in solutions of osmotic pressure below that of the blood. A graphic representation of a typical experiment is given in figure 1.

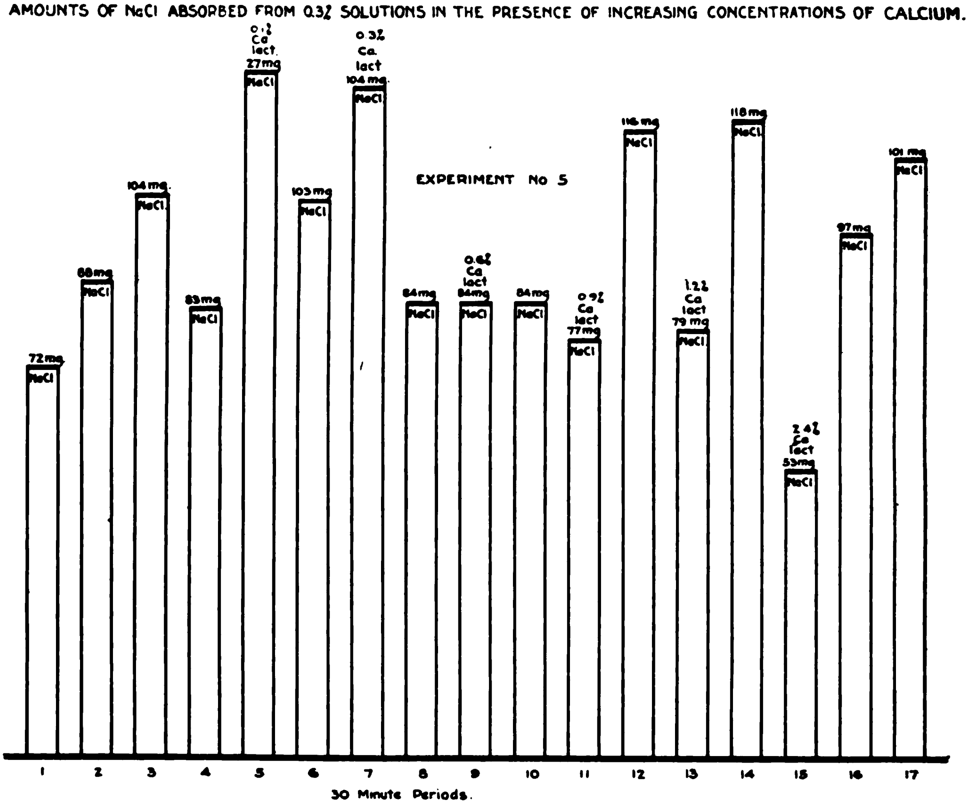
AMOUNTS OF NaCl ABSORBED FROM 0.3% SOLUTIONS IN THE PRESENCE OF INCREASING CONCENTRATIONS OF CALCIUM.

Fig. 1

The fluid absorption may, at first, show a slight increase over the control periods. This is indicated in period 1 of experiment 4 and in periods 1 and 2 of experiment 5. This occurs coincidently with the increased chloride absorption. Thereafter there is a progressively decreasing fluid absorption, from the experimental solutions, with increasing concentrations of calcium lactate.

During absorption the concentration of sodium chloride in the control periods is always increased. On the other hand, when calcium lactate is present the concentration of chlorides decreases. This is due to the fact that chlorides are absorbed more rapidly than the water, even though the total absorption may be inhibited. This is observed in periods 2, 3, 4, 5 and 6 of experiment 5. With low concentrations of calcium lactate the effect just described is less pronounced. This same phenomenon is noted when sodium sulphate is added to sodium chloride in the colon.

A lag effect, analogous to that with sodium sulphate in the intestine, is also observed after calcium lactate has been present in the intestine. This is evidenced by an increased chloride absorption from solutions of sodium chloride in the control periods. It is especially noticeable in experiment 4. There is, furthermore, an indication that, after the high concentration of calcium lactate, the chloride absorption in the control periods may return to the normal level (exper. 4, period 7). In experiment 5, when the intestine was washed, after each introduction of calcium lactate, the general course just described is also evident, but the figures vary too much for comparison.

In the last column of the table has been compiled the ratio of calcium to chlorine. It will be noticed that, although the concentrations of calcium used in some of the experiments present wide gaps, the ratio at which the absorption of chlorides begins to be inhibited shows a striking constancy, namely:

Experiment 2	Period 5–6,	Ca: Cl = 1: (2.3–1.5)
Experiment 3	Period 6–7,	Ca: Cl = 1: (1.4–0.8)
Experiment 4	Period 2	Ca: Cl = 1: 1.0
Experiment 5	Period 4	Ca: Cl = 1: 1.1

This relationship is the more remarkable when it is considered that the concentration of sodium chloride in experiments 2 and 3 is nearly twice that of the subsequent experiments. This point will receive further investigation.

In order to demonstrate the action of another calcium salt, in table 2 is presented an experiment in which the calcium was introduced in the form of calcium chloride. The following points are evident; less chlorides are absorbed from the same percentage and amount of chlorides in the form of calcium chloride than from sodium chloride. The latter was introduced into the colon in period 1 and the former in period 2. In a mixture of sodium chloride and calcium chloride (period 4), of the same chloride content, much less chlorides are absorbed than from sodium chloride alone.

TABLE 2

The effect of Ca *upon water and chloride absorption in the colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME FLUID − LOSS	NaCl INTRODUCED	Cl AS $CaCl_2$ INTRODUCED	NaCl RECOVERED	CHANGE NaCl + GAIN − LOSS	CHANGE NaCl − LOSS	RATIO Ca : Cl
	min.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*	
Experiment 1										
Period 1...	60	87	34	−53	0.33	0	0.48	+0.15	−124	
Period 2...	60	85.5	43	−42.5	0	0.34	0.54	+0.20	− 59	1:1.8
Period 3...	60	87	42	−45	0.31	0	0.45	+0.14	− 81	
Period 4...	60	85.5	43.5	−42	0.32	0.32	0.49	+0.17	− 60	1:1.0

DISCUSSION

The above data show that calcium salts affect the absorption of chlorides from sodium chloride in two stages. First: with low concentrations of calcium lactate, there is a speeding effect similar to that which we obtained with sodium sulphate on sodium chloride. Second: with still higher concentrations of the calcium lactate, the absorption of chlorides is inhibited. As might be expected, with an intermediate concentration of calcium lactate a balanced action is noted; that is, a point is reached where the chloride absorption, from the mixture of sodium chloride and calcium lactate, is of the same grade as from pure sodium chloride solution.

All of these stages may take place below a Δ iso-osmotic with the blood. On the other hand, the speeding effect of sodium sulphate persisted throughout the whole range of Δ.

When the calcium lactate is introduced with sodium chloride, there is a reversal of the normal behavior of sodium chloride of equal concentration in the colon. That is, the percentage concentration of hypotonic solutions (0.3 per cent) is reduced instead of increased. An effect similar to that of the chloride in mixtures of sodium chloride and sodium sulphate.

There is an indication that calcium chloride behaves similarly to calcium lactate. On the basis of the ideas of Clowes (5), it is probable that the phenomena described are concerned with the predominant cation calcium and anion chlorine, an indication of which is the rather constant ratio of Ca: Cl at the point where the inhibitory action is first exerted.

There is evident in the above experiments a "lag" effect, since at one point there is an increased absorption of chlorides over the control periods which later (after the inhibitory effect) returns to about the normal level. When the colon is thoroughly washed with salt solution after each introduction of the calcium lactate this "lag" effect is still evident.

It has been shown in the preceding paper that sodium sulphate in all concentrations speeds the absorption of sodium chloride. We suggested that the effect may be related to osmotic factors alone. With calcium lactate, however, it is at once apparent that no such simple explanation is adequate. For the stage of inhibition, at least, an effect upon the permeability of the intestinal wall would seem at present to be the most probable cause of the phenomenon.

CONCLUSIONS

1. Calcium lactate in increasing concentrations first accelerates, then inhibits the absorption of chlorides from solutions of sodium chloride in the colon.
2. There is an indication that this action bears a relationship to the ratio of calcium to chlorine.
3. The first stage of the action of calcium lactate upon sodium chloride resembles that of sodium sulphate upon that salt. The last stage presents the opposite effect to that of sodium sulphate.

BIBLIOGRAPHY

(1) Loeb: Science, N. S., 1910, xxxiv, 653, and others in same publication. Proc. Soc. Exper. Biol. and Med., 1916, xiv.
(2) Osterhout: Science, N. S., 1911, xxxiv, 187; 1912, xxxv, 112; 1916, xliv, 395; and other papers in same publication.
(3) Lillie: This Journal, 1911, xxxvii, 289.
(4) Mathews: This Journal, 1905, xii, 419.
(5) Clowes: Science, N. S., 1916, xliii, 750.
(6) MacCallum: Univ. of Cal. Pub. Physiol., 1905, ii, 87, 93.
(7) Davis: Journ. Urology, 1917, i, 113.
(8) Goldschmidt and Dayton: This Journal, 1919, xlviii, 419.

AN EXPERIMENTAL CONTRIBUTION TO THE BIOLOGICAL RELATION OF THE DIASTASE-FERMENT BETWEEN THE MATERNAL BLOOD, THE FETAL BLOOD AND THE AMNIOTIC LIQUID OF ANIMALS

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As to the questions whether the change, natural or artificial, produced in the maternal blood has any influence upon the fetal blood or not, and under what law, if any, it acts upon the latter, there has been little investigation as yet. These questions, however, arouse great interest in practical medicine as the change may interfere with the embryonal metabolism, and consequently it may sometimes lead to the death of the fetus, causing an abortion or premature labor. The relation between the maternal body and the fetus and amniotic liquid means, it must be admitted, the metabolism in the placenta on one hand, and a certain function of the amniotic epithelium on the other. Few conclusive evidences have ever been given in the investigation of the function of the placenta, and accordingly the present paper will serve as an experimental proof of the general view.

That organic and inorganic substances pass into the fetal blood has been shown by experiments and published by many investigators, of which the following are the chief points that have important relations to my investigation:

Fat (which was most successfully investigated by Costa (1), Bondi (2), and Oshima (3)), has been observed to pass into the fetus not as such, but in a modified form; besides, carbohydrates (Bang (4) and Varaldo (5)), anti-bodies (Mounn af Heurlin (6)), antitoxin (Polano (7)), and haemolysine (Kreidl and Mandl (8)) are also ascertained to pass into the fetus; with regard to proteins, however, it was shown by Ascoli (9) that they do not pass through the placenta but that the latter acts as a digestive organ for them. Kreidl and Mandl's experiments, which demonstrated the passage of haemolysine into the maternal body from the fetus, show as just stated, that haemolysine passes through the placenta.

The results of the various investigations hitherto made suggest the idea that the placenta, being an important barrier for the fetus, does not allow the free passage of anything, but serves as an alimentary and life-protecting organ of the fetus; moreover from its behavior toward glycogen (as shown by Lochhead and Cramer (10)), we may say that the fetus takes up material necessary for its development quite independently of the nutrition of the maternal body.

An interesting question which has presented itself in recent years in connection with the maternal body, the fetus and the amniotic liquid is the relation of the pigment of living bodies among these three. With regard to this question we have not yet come to a conclusive opinion, but it seems certain that coloring-matters usually do not pass through the placenta. No researches have yet been published whether a ferment will pass through the placenta and although the passage of glucose is an established fact, its quantity and its relation to the amniotic liquid have not been fully investigated by any one. I was therefore engaged in the present researches in order that I might make a contribution to the knowledge in this direction.

For my experiments I selected diastase as it is a ferment that can be accurately determined, and the object of my investigation was to know the result of the injection of diastase into the maternal body, that is to say, if the increase of diastase in the maternal blood by any means had any influence upon the fetus and the amniotic liquid. In short, it was my intention to ascertain the permeability of the placenta to the ferment. Besides, I entertained some hope that I should be able to have some glimpses of the origin of the amniotic liquid by observing its attitude toward the ferment, but to my disappointment, I could not obtain sufficient data from my experiments to throw light on the origin of this fluid. I also tried to compare the transudate in the abdominal cavity with the amniotic fluid, but as it was very difficult to obtain the latter—fair amounts may be obtained in rabbits but guinea pigs have little, if any—I excluded it from the present researches. With the above objects in view, therefore, I made the following experiments.

In the first place, I examined whether diastase would pass into the blood of the fetus by producing a retention of diastase in the maternal body by various means (such as tying the renal blood vessels and the injection of uranium).

In the second place, I examined the change of quantity of the diastase-ferment when it was artificially injected into the blood vessels, and at the same time injecting glucose, which is certainly known to pass, determined its quantity and compared it with the quantity of the ferment that passed.

In the third place, having found that when diastase was injected into the blood of an animal the greater part of it soon disappeared, I studied the injection of various other ferments in order to find the reason for the disappearance of diastase and compared these results with similar ones obtained from experiments with animals not yet impregnated.

Animals used in the experiments. Guinea pigs are best fitted for the study of diastase as its value is high in these animals, and in all my experiments I used guinea pigs fed with ordinary food, selecting bigger ones as much as possible. Of the pregnant ones I selected those which were at the period of from forty to fifty days of their pregnancy, for at an early period the fetal blood is too small in quantity, and on the other hand the amniotic liquid becomes less as the time of delivery approaches (the duration of pregnancy of the guinea pig is about nine weeks).

Methods used in drawing the blood and the amniotic liquid. The maternal blood was taken into a sterile centrifuge tube from the carotid artery. For the fetal blood, after abdominal incision the uterus was exposed and opened and the placenta separated without injury to the fetal sac. The amniotic liquid was next drained into a sterile beaker, the fetus was then carefully wiped with sterile gauze after which the fetal blood was drawn from an incision in the neck, care being taken to avoid contamination with cerebro-spinal fluid. Care must also be taken to prevent the mixture of the liquid of the extra-body cavity with the amniotic liquid, for the amniotic sac of the rabbit and the guinea pig, unlike that of man and other higher animals, retains the yolk-sac around it until the last stage of the embryonic life; for this reason it is advisable to seize the amniotic sac with the cord downward, for then the liquid will gather around the cord after which a small hole in the region of the head permits the amniotic liquid to be poured out without contamination.

Notes on the determinations of diastase. I adopted the method of Wohlgemuth (11) in determining diastase. From earlier work I found that this method was not reliable unless everything used for it was disinfected, and accordingly in my present investigation all the instruments, pipettes, beakers and test-tubes were subjected to 150°C. in the dry heat sterilizer, and all the salt solution and starch solution were also sterilized beforehand.

The maternal blood was diluted one hundred times its volume, and the fetal blood and the amniotic liquid ten times their volumes with sterile 0.85 per cent saline solution, 1.0 cc. of each was taken into test-tubes, after which they were all again doubly diluted with common salt water. As to the starch solution, I used 0.2 per cent solution of soluble starch prepared by the Kahlbaum Company, adding 5.0 cc. to each test-tube. The tubes were carefully examined after they had been kept in a 37°C. incubator for twenty-four hours.

As an indicator a $\frac{N}{50}$ iodine-solution was used.

EXPERIMENTS ON THE DIASTASE IN THE MATERNAL BODY, THE FETUS AND THE AMNIOTIC LIQUID DURING THE RETENTION OF DIASTASE IN THE MATERNAL BLOOD

a. Determination of the diastase in the maternal blood, the fetal blood and the amniotic liquid of a pregnant guinea pig. The diastase content of the blood of the pregnant guinea pig is already known, but no one has ever determined that of the fetus and of the amniotic liquid at the same time; so I determined them preliminary to my experiments and used them as a control for my subsequent experiments. The diastase was always determined as just described. In the case of blood the serum was used for test.

The diastase value of the maternal blood in the table is from 400.00 to 800.00, the average being 602.08.

The diastase in the fetus has variable values between the two extremes of 40.00 and 322.58, the average being 109.29 for the blood and 80.31 for the amniotic fluid.

The diastase both in the fetal blood and in the amniotic liquid is remarkably small as compared with that of the maternal blood, being only one-fifth to one-sixth of the latter.

Bial (12), who investigated the diastase value in the embryonic stage of man and in the fetus of animals, also recognized a trace of diastase in the blood of the fetus. Of the ferments in the amniotic liquid we have Bondi's (13) report. According to him we have pepsin, fibrin-ferment and diastase always in the liquid, but no other ferments, and there is no doubt that we have diastase in the amniotic liquid.

b. Experiments on the change of the quantity of diastase in the maternal blood, and on its passage into the fetus after the renal blood vessels of a pregnant guinea pig have been tied. As the diastase-ferment is always present in urine, if we tie the renal blood vessels on both sides, it is probable that a retention of diastase would occur in the maternal body. To test this point the following experiment was performed on a pregnant guinea pig with particular reference to the diastase content of the fetus. Under light ether anesthesia the kidneys were exposed by dorsal incisions. Ligatures were tied about the artery, vein and ureter on either side and the abdominal incision closed. The procedure occupied less than five minutes. In no case did the animal live more than twenty-four hours, and many of the feta were found dead when observations were made three to six hours after the operation.

TABLE 1

NUMBER	BODY WEIGHT	DAYS OF PREGNANCY	DIASTASE OF THE		
			Maternal blood	Fetal blood	Amniotic liquid
	grams				
1	490	About 50	D=416.66		
2	580	About 50	400.00	D=40.00	
3	620	More than 40	400.00	80.00	
4	850	More than 50	800.00	161.28	
5	900	More than 40	800.00	40.00	D= 40.00
6	720	More than 40	400.00	40.00	40.00
7	700	More than 50	800.00	322.58	80.00
8	680	More than 50	800.00	80.00	161.29
Average.....			602.082	109.122	80.31

Diastase was determined by the same method as before. The diastase content of the maternal blood varies from 800.00 to 3225.80, averaging 1250.177.

When compared with those of the control (table 1), we see that the minimum here corresponds with the maximum of the control and that the maximum is four times as great after ligation of the renal vessels than normally. Besides, the individual cases in the table evidently show an increase of diastase in the maternal blood and that the diastase content in the fetal blood, which is 80.00 to 322.58, with the average of 161.13, is somewhat greater than 109.122, which is the average diastase value of the fetus in the control experiments. If no. 7

in the control series, which deviates widely from the average, be omitted the average becomes 73.54. Using this figure as the control value the ligation experiments show a twofold increase in fetal blood diastase. It appears therefore that the increase in the diastase content of the maternal blood as the result of ligation of the renal vessels is responsible for the increase in diastase content of the fetal blood.

In the present experiments I had six cases of dead fetus, all of which were examined shortly after their death. The relation between death and the quantity of diastase may depend upon the time that elapses after the death, and in my small number of experiments I found that there was little relation between them, that is to say, in some feta still alive I found little diastase while in some dead ones there was a large quantity of it, and thus it pointed to the negation of any definite relation between them. The operation of tying the blood vessels of the kidneys must be a very great stimulus and it is possible that such a stimulus might cause the increase of the ferment. However since diastase determinations made shortly after the operation was completed never showed an increase over the control and usually showed a decrease, I believe this factor is of no significance.

TABLE 2

NUMBER	BODY WEIGHT	DAYS OF PREGNANCY	TIME AFTER OPERATION	DIASTASE OF THE		FATE OF FETAL LIFE
				Maternal blood	Fetal blood	
	grams					
1	700	More than 50	6°0′	D = 800.00	D = 161.29	Dead
2	750	More than 50	5.40	800.00	161.29	Dead
3	640	More than 50	4.0	1612.90		Dead
4	760	More than 50	3.40	3225.80	322.58	Living
5	760	More than 50	3.30	800.00	80.00	Dead
6	800	More than 50	3.0	800.00	161.29	Dead
7	700	More than 40	3.0	800.00	80.00	Living
8	740	More than 50	4.0	800.00	161.29	Living
9	700	More than 50	4.0	1612.90	161.29	Dead
Average.				1250.177	161.13	

c. The relation of diastase in the case of nephritis caused by the injection of uranium. It is known that the injection of uranium gives rise to nephritis, producing a lesion in the convoluted tubule in particular. I made this experiment in order to see if nephritis produces any change in the quantity of diastase in the maternal blood or in the fetal blood and amniotic liquid.

Treatment. From three to eight days after the subcutaneous injection of a 0.1 per cent solution of uranium acetate into the abdominal wall of the animal in the proportion of 1 cc. per kilogram of the body weight, I took the blood and the amniotic liquid and examined them. Before the experiment I always ascertained the presence of albumin in the urine (albuminuria) and measured its quantity by Sueyoshi's (14) albuminometer.

The diastase in the maternal blood is 800.00 to 6250.00, averaging 1901.61. No. 3, however, deviates widely from the average. If this determination be omitted, the average becomes 1736.08. Even this average shows a great increase when compared with that of the control.

TABLE 3

NUMBER	BODY WEIGHT	DAYS OF PREGNANCY	TIME AFTER INJECTION	QUANTITY OF ALBUMIN	DIASTASE OF THE			FATE OF FETAL LIFE
					Maternal blood	Fetal blood	Amniotic liquid	
	gms.		*days*	*per cent*				
1	680	More than 50	4	1.5	D=3225.80	D=322.58		Dead
2	700	More than 40	2	2.0	1612.90	Dead		Dead
3	580	More than 50	3	3.0	6250.00	Dead	D=80.00	Living
4	900	More than 50	3	1.5	1612.90	161.29		Living
5	860	More than 50	3	0.5	800.00	80.00		Living
6	740	More than 50	4	0.5	1612.90	322.58		Living
7	770	More than 50	4		800.00	80.00	40.00	Living
8	720	More than 50	8	2.0	800.00	40.00	80.00	Living
9	840	More than 50	4	0.5	400.00	161.29	80.00	Living
Average.					1901.61 (1736.08)	166.82	65.00	

The diastase in the fetal blood is 80.00 to 322.58, average 166.82, thus showing a slight increase as in the preceding case. The diastase value of the amniotic liquid being 40.00 to 80.00, averaging 65.00, shows no noteworthy tendency of increase.

As indicated in the table (table 3), the minimum of the diastase value of the maternal body corresponds with the highest of the control, and the maximum is seven times as large as that of the latter. The individual cases all clearly show an increase, indicating that there is a retention of diastase in the maternal body. In the fetus we find some increase, but there is no influence whatever upon the amniotic liquid. If there is any relationship between the quantity of diastase retained and the quantity of albumin or the length of time after the injection, my small number of experiments failed to show it.

From the two preceding experiments, we may conclude that when diastase is retained in the maternal blood, the diastase ferment passes into the fetal blood. There is, however, no evidence of its passage into the amniotic liquid. This indicates the passage of the ferment through the placenta and is undoubtedly dependent upon the physical permeability of the organ.

THE DIASTASE AND THE BLOOD-SUGAR IN THE MATERNAL BLOOD, FETAL BLOOD AND AMNIOTIC LIQUID AFTER AN ARTIFICIAL INJECTION OF DIASTASE AND GLUCOSE

a. The determination of the blood-sugar in the maternal blood, fetal blood and amniotic liquid of a pregnant guinea pig. The determination of sugar in the maternal blood, fetal blood and amniotic liquid of a pregnant guinea pig has not been reported. This is probably due to the fact that the quantity of the fetal blood and the amniotic liquid is so small that the method of determining sugar hitherto in use is impracticable with them.

TABLE 4

NUMBER	BODY WEIGHT	DAYS OF PREGNANCY	SUGAR OF THE		
			Maternal blood	Fetal blood	Amniotic liquid
	grams		*g/dl.*	*g/dl.*	*g/dl.*
1	900	More than 40	0.1110	0.0633	0.0751
2	720	More than 40	0.0869	0.0462	0.0820
3	700	More than 50	0.1099	0.1199	0.0655
4	680	More than 50	0.1435	0.0433	0.0767
Average.......			0.11282	0.06817	0.07432

Bang's (15) micro-method being best suited for the present investigation, I soaked four pieces of blotting-paper prepared after Bang's method in the bloods, and two other pieces in the amniotic liquid. The diastase was determined as before. For the determination of sugar I used potassium chloride with acidity, which was Bang's original method, and for titration a $\frac{N}{200}$ iodine solution.

The blood-sugar of the maternal blood is 0.0869 to 0.1435 g/dl., averaging 0.11282 g/dl.

The blood-sugar of the fetus is 0.0462 to 0.1199 g/dl., averaging 0.06817 g/dl.

The sugar in the amniotic liquid is 0.0655 to 0.0767 g/dl., averaging 0.07432 g/dl.

The quantity of sugar in the maternal blood, like that of man and other animals, seems to be of the value of about 0.1 g/dl. but in the fetus and in the amniotic liquid it is very much less.

TABLE 5

NUMBER	BODY WEIGHT	DAYS OF PREGNANCY	QUANTITY OF INJECTION	CONCENTRATION OF SOLUTION DIASTASE	TIME OF BLOOD DRAWING	DIASTASE OF			SUGAR OF		
						Maternal blood	Fetal blood	Amniotic liquid	Maternal blood	Fetal blood	Amniotic liquid
	gms.		cc.	per cent							
1	1070	More than 60	1.0	1.0	15′-20′	D= 200.00	D= 161.29		0.1677	0.0991	
2	900	More than 55	1.0	1.0	15′-20′	400.00	161.29		0.1820	0.1092	
3	770	More than 55	1.0	1.0	20′-25′	400.00	161.29		0.1132	0.1433	
4	950	More than 50	3.0	1.0	20′-25′	800.00	80.00		0.2019	0.0595	
5	900	More than 50	2.0	0.5	20′-25′	800.00	80.00	80.00	0.1572	0.1133	0.0715
6	1200	More than 50	4.0	0.5	20′-25′		161.29	161.29	0.1094	0.1045	0.0783
7	800	More than 50	4.0	0.1	30′-35′	800.00	80.00	322.58	0.1895	0.1411	0.0567
8	900	More than 50	7.0	0.1	20′-25′	400.00	80.00	161.29	0.2006	0.1713	0.0530
9	740	More than 50	3.0	0.5	5′	800.00	80.00	80.00	0.3357	0.1174	0.0558
10	650	More than 50	3.0	0.5	5′	800.00	161.29	80.00	0.2120	0.0821	0.0782
11	690	More than 50	3.0	0.5	5′				0.2536	0.1499	0.0461
12	720	More than 50	3.0	0.5	5′				0.2712	0.1711	0.0499
Ave.						600.00	121.895	147.29	0.19950	0.13015	0.06118

b. The diastase and the sugar in the maternal blood, fetal blood and amniotic liquid after the injection of Taka-diastase and glucose. I made the following experiments in order to study the influence of a ferment upon the maternal body, the fetus and the amniotic liquid when it was injected into the maternal body parenterally and also to compare the result with that of a substance which is already known to pass. I used Taka-diastase for the ferment and glucose (Merck) for the substance with which to compare it.

Method of experiments. I dissolved Taka-diastase in the proportion of 0.1, 0.5 and 1 per cent in a 0.85 per cent salt solution, and after neutralizing it with a $\frac{N}{10}$ soda solution, added pure glucose in the proportion of 10 per cent. This solution was injected into a pregnant guinea pig through the vein on one side of the neck with Pravatz's injector under a very slight pressure, for when the solution is quickly injected, it causes various disorders in the body of the animal, but if it is injected very slowly, the animal is able to stand a large quantity of it in considerable concentration. The blood of guinea pigs injected with different quantities of the solution is taken at intervals of time for the determination of the diastase and glucose in the maternal blood, in the fetus and in the amniotic liquid. The table shows that the diastase of the maternal blood is from 200.00 to 800.00, averaging 600.00. The diastase of the fetus is 80.00 to 161.29, the average being 120.64. The diastase of the amniotic liquid is 80.00 to 322.58, averaging 147.52. The blood-sugar of the maternal body is 0.1094 to 0.3357, averaging 0.19950 g/dl., that of the fetus is 0.0595 to 0.1713, the average being 0.13015 g/dl.; that of the amniotic liquid is 0.0567 to 0.0783, averaging 0.06128 g/dl. Thus although a diastase-ferment is artificially injected into the blood stream, no change of quantity of diastase takes place in the maternal blood.

In the fetal blood, however, the average shows a slight increase, and when we look at the individual cases, we find that the most of them fall close to 161.29, which shows an increase of diastase in the case of the fetal blood. In the case of the amniotic liquid there is also an apparent increase of diastase.

Sugar increases both in the maternal and the fetal blood, and since we see that both diastase and sugar, even in the case of an artificial parenteric injection, produce its increase in the fetal blood as in the case of the autoretention, it is certain that the ferment, like sugar, passes through the placenta.

In the amniotic liquid we recognize a little increase of the ferment, but no passage of sugar into it. This fact is difficult to explain.

In the case of an artificial introduction of diastase into the body, unlike the case of the autoretention of the ferment, I found a singular phenomenon in that there was no increase of diastase in the maternal blood even though a pretty large quantity of diastase was injected into it. There are only three possible explanations for this phenomenon: *a*, a regulation may take place in the placenta and the diastase in the maternal blood may be always adjusted by it; *b*, the ferment may soon disappear on account of its toxic influence even if it be introduced in large quantity into the body; or *c*, it may be due to a regulation that may take place in some part of the maternal body.

I shall again touch these questions in the following chapter. As regards the concentration and the quantity of the solution and the length of time that intervenes between the injection and the drawing of blood, I may say that they have no relation to the quantity of diastase though my experiments are few in number.

When sugar is passed directly into the vein, unlike the sugar that is absorbed by the alimentary canal, it is not affected by any digestive ferment or by the action of the liver, but enters the system of the general circulation directly. According to Bang's (15) experiments, the greater part of the injected glucose undergoes transformation and disappears at once while a part of it is deposited in various organs or is excreted by the kidneys, and for this reason the glucose that appears in the measurement is very small in quantity when compared with the injected glucose which is as much as 10 per cent. According to Bang, moreover, the blood-sugar reaches the acme of its quantity five minutes after its intravenous injection and for thirty minutes after that it gradually decreases. It was this observation which led me to select the time shown in the table for drawing the blood in my experiments.

As regards the question whether the quantity of injected grape-sugar has anything to do with blood-sugar, my experiments are too few to settle it.

DETERMINATION OF DIASTASE AFTER THE INJECTION OF TAKA-DIASTASE, TRYPSIN AND PEPSIN

We have learned from the preceding experiments that the diastase in the maternal blood does not increase if we introduce it artificially into the maternal body, and I have made some suggestions to explain this fact. Here I propose to take up the question whether or not the diastase is affected by the placenta, and also the possibility that the diastase originally present in the blood may be destroyed by the toxic influence of the added ferment, the injected diastase replacing it so that the circulating diastase remains unchanged. The third supposition that the diastase may be directly adjusted by a regulation that may be taking place in some part of the maternal body is a complex question and will not be considered at the present time.

According to Oppenheimer (16), ferments exert little toxic influence on the body, while Hildebrandt (17) supposes that the toxic influence is due to the albumose or the amino-acid, etc., contained in it. Wishing to see if ferments really had no toxic influence, I injected various ferments into animals and observed the change of the blood-diastase, for the presence or absence of a toxic influence can be inferred from the change produced on the diastase. The guinea pigs used in the present experiments were all non-pregnant ones, for if the injection of ferments into non-pregnant animals produces the same change as in a pregnant one, it will at once prove that the change has nothing to do with the placenta.

The ferments used in the present experiments were Taka-diastase, trypsin (prepared by the Grübler Company), and pepsin (Sankyo Company).

It was necessary therefore to establish a control for this new technique. The results obtained by this method are given in table 6.

a. Determination of the blood-diastase of non-pregnant guinea pigs. In the following experiments, unlike the foregoing determination of diastase, the serum was not diluted and the starch solution was of 1.0 per cent.

TABLE 6

NUMBER	BODY WEIGHT	DIASTASE
	grams	
1	450	D = 312.50
2	450	312.50
3	500	625.00
4	550	312.50

The blood-diastase of non-pregnant guinea pigs varies between 156.25 and 625.00.

b. Determination of diastase after the injection of Taka-diastase. For injection a 0.5 per cent solution of Taka-diastase in physiologic salt solution neutralized with a $\frac{N}{10}$ soda solution was prepared. This was injected into the vein as before, and the difference of the diastase value was likewise observed by drawing the blood at different times and after the injection of different quantities. The method of determination was the same as before.

The blood-diastase of the animals injected with Taka-diastase shows no difference from that of the ordinary animal, and it is also quite independent of the quantity injected and of the time of drawing the blood.

TABLE 7

NUMBER	BODY WEIGHT	CONCENTRATION OF SOLUTION	AMOUNT OF INJECTION	TIME OF BLOOD DRAWING	DIASTASE	CONDITION AFTER THE INJECTION
	grams	*per cent*	*cc.*			
1	340	0.5	2.0	1′	D=312.50	No particular symptoms of poisoning
2	400	0.5	4.0	Immediately after	312.50	No particular symptoms of poisoning
3	300	0.5	3.0	2′	312.50	No particular symptoms of poisoning
4	360	0.5	3.6	5′	156.25	No particular symptoms of poisoning
5	400	0.5	4.0	10′	312.50	No particular symptoms of poisoning
6	380	0.5	3.8	30′	312.50	No particular symptoms of poisoning

TABLE 8

NUMBER	BODY WEIGHT	CONCENTRATION OF SOLUTION	AMOUNT OF INJECTION	TIME OF BLOOD DRAWING	DIASTASE	CONDITION AFTER THE INJECTION
	grams	*per cent*				
1	380	1.0	0.95	5′	D=312.50	No particular symptoms
2	340	1.0	1.7	5′	312.50	No particular symptoms
3	350	1.0	3.5	5′	625.00	No particular symptoms
4	420	1.0	3.2	5′	312.50	No particular symptoms
5	320	2.0	3.2	5′	625.00	Uneasy

TABLE 9

NUMBER	BODY WEIGHT	CONCENTRATION OF SOLUTION	AMOUNT OF INJECTION	TIME OF BLOOD DRAWING	DIASTASE	CONDITION AFTER INJECTION
	grams	*per cent*				
1	360	1.0	1.8	5′	D=400.00	No particular symptoms
2	360	1.0	3.6	5′	800.00	No particular symptoms
3	380	1.0	3.8	5′	312.50	No particular symptoms
4	360	2.0	3.2	5′	312.50	No particular symptoms
5	420	2.0	4.2	5′	161.29	Uneasy, palpitation of the heart, suspension of respiration, blood-flowing, dark color

c. Determination of diastase after the injection of trypsin. The experiment was carried on in the same manner as the preceding, and the solution of trypsin was of 1.0 to 2.0 per cent. Though I found in the preceding experiment that the time of drawing the blood and the quantity injected had little relation to the blood-diastase, I injected different quantities of trypsin as shown in the table, with the hope that different kinds of ferments might produce different results. The trypsin was also neutralized with a $\frac{N}{10}$ soda solution.

The result of the injection is the same as the preceding, being quite independent of the quantity injected and showing no difference from that of the ordinary animal.

d. Determination of diastase after the injection of pepsin. The concentration of the pepsin liquid (a solution of pepsin in physiologic salt solution) was from 1.0 per cent to 2.0 per cent. After neutralizing it with a soda solution as before, it was injected into the blood vessel in the proportion of 0.5 to 1.0 per kilo.

As in the two preceding cases, the quantity of diastase remains unchanged, being quite independent of the concentration of the pepsin-liquid and the quantity injected.

Experiments were also made by injecting larger quantities of these ferments, but in such cases the animals either died or presented serious symptoms, and accordingly they are all excluded from the tables as it is not proper to take the quantities of diastase of such cases into account.

According to Hildebrandt (17), the lethal quantity of diastase, pepsin etc., for a rabbit of medium weight is 0.1 gram, and according to Ishihara's (18) experiments, if more than 0.07 gram of trypsin is injected in the vein, anaphylactic shock is produced in two cases out of three. In my experiments therefore the quantity of ferments injected into the animal body was kept within such limits that it would not produce these symptoms, and only those animals which presented such slight symptoms as palpitation of the heart, a little difficulty of respiration, etc., as given in the tables, were subjected to the examination.

As we see in the present experiments, the diastase introduced parenterally into the body also disappears at once even in the cases in which the placenta is not concerned, which shows that it has nothing to do with the placental regulation. Nor can we consider it due to a toxic influence of the ferment, for if there is such an influence we should expect a noticeable change in the diastase in the experiments. The disappearance, therefore, of the diastase parenterally injected into the animal body without producing any increase of the blood-diastase must be explained by the supposition that there is an arrangement in the body by which the injected diastase is destroyed at once.

According to Hildebrandt's investigation, the ferment is a blood-toxin which reduces haemoglobin. We cannot, however, tell the fate of the ferment until we settle the question whether it, like sugar, disappears at once or is taken up by the liver, pancreas, muscles, etc., or whether it is destroyed by some glandular secretion or is excreted in urine, and as it is not directly connected with my present experiments nor has my investigation of it yet come to completion, I will not deal with it here.

SUMMARY

1. When the blood vessels of the kidneys are tied or when nephritis is caused by the injection of uranium, a retention of diastase in the maternal blood is produced and there is an increase of diastase in the fetal blood.
2. In the case of nephritis after the injection of uranium, there is no change in the quantity of diastase in the amniotic liquid.
3. When Taka-diastase and pure glucose are injected simultaneously into the vein, blood-sugar increases both in the maternal and fetal bodies, but no change is produced in the amniotic liquid and there is no change in the diastase of the maternal blood, but we find a little increase of it in the fetus and in the amniotic liquid.
4. From the above facts we are entitled to say that the placenta is permeable to the diastase-ferment.
5. We cannot explain the reason why the diastase-ferment passes into the amniotic liquid while sugar does not.
6. In the case of the parenteral injection of a ferment it is lost in the animal body, which seems to be due not to the toxic influence of the ferment or to the regulation of the placenta, but to the regulating function of some other part of the body.
7. As regards the origin of the amniotic liquid, there are many complicated relations, and it is difficult to explain it by the simple results of the present experiments.

In concluding this paper I wish to express my deep thanks to Professor Doctor Katayama, who kindly permitted me to pursue my investigation in his department, and to Professor Doctor Mita, who kindly guided and advised me throughout this investigation.

BIBLIOGRAPHY

(1) **Costa**: Ann. d. Obst. et Ginec., no. 7 (Ref. Biochem. Zeitschr., Bd. iii).
(2) **Bondi**: Arch. f. Gynec., 1911, xciii.
(3) **Oshima**: Zentralbl. f. Physiol., 1907, Bd. xxi, no. 10.
(4) **Bang**: Die Blutzucker, 1913.
(5) **Varaldo**: Arch. d. Obst. et Ginec., 1905 (Ref. Biochem. Zeitschr., Bd. iv).
(6) **Mounn af Heurlin**: Arch. mens. d'obst. et d. gynec. Juin. (Ref. Frommel's Jahres Ber. f. G. and G., 1913).
(7) **Polano**: Zeitshcr. f. G. and G., Bd. liii and Ref. Muenchen. med. Wochenschr., 1904, no. 42.
(8) **Kreidl and Mandl**: Wiener klin. Wochenschr., 1904, no. 22.
(9) **Ascoli**: Zeitschr. f. physiol. Chem., Bd. xxxvi, 516.
(10) **Lochhead and Cramer**: Journ. Physiol., 1907, xxxv.
(11) **Wohlgemuth**: Grundriss der Fermentmethoden, 1913; Biochem. Zeitschr., 1908, Bd. ix.
(12) **Bial**: Pflüger's Arch., Bd. liii.
(13) **Bondi**: Zentralbl. f. Gynec., 1903, no. 21.
(14) **Sueyoshi**: Mitteil d. mediz. Gesellsch. zu Tokyo, Bd. xxviii.
(15) **Bang**: Die Blutzuker, 1913.
(16) **Oppenheimer**: Die Fermente, 1913.
(17) **Hildebrandt**: Virchow's Arch., Bd. cxxi and cxxxi.
(18) **Ishihara**: Mitteil. d. mediz. Gesellsch. zu Tokyo, Bd. xxix.

THE SYNERGY AND ANTAGONISM OF SODIUM SALTS IN BARIUM STIMULATION

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The stimulating power of the sodium salts and the necessity of these salts for the activity of most contractile structures have long been recognized. Ringer (1) observed the rhythmical contractions of a muscle placed in a sodium chloride solution. Lingle (2), (3) showed that without sodium salt the heart beat is impossible, and the rhythmical activity of the jellyfish was by Loeb (4) also ascribed to this salt.

Concerning the stimulating effect of barium chloride Ringer (1) noted that its power to produce contractions and persistent spasm much exceeds that of the saline solutions and that the previous application of a saline solution increases the stimulating effect of subsequently applied barium. Overton (5) found that a muscle which had been treated with a 6 per cent cane sugar solution for a sufficient length of time failed to respond to barium stimulation; he attributed this to the lack of sodium salts in the sugar-treated muscle. This I (6) corroborated by showing that if a muscle is bathed with a 6 per cent cane sugar solution for twenty minutes, the subsequent application of m/80 barium chloride in 6 per cent cane sugar produced a few feeble contractions only after a latent period of eighteen minutes; a normal muscle in the same concentration of barium chloride in a m/8 sodium chloride solution begins to show violent twitches in from one to five minutes. Such a sugar-treated muscle placed in a 0.7 per cent sodium chloride solution speedily regains its normal irritability toward barium. The loss of irritability which a tissue experiences during its stay in a solution of a non-electrolyte is generally said to be due not so much to the specific action of the particular non-electrolyte as to the diffusion of the necessary salts from the irritable structure into the non-electrolyte (7). Hence, the presence of sodium salt in the muscle is indispensable for the stimulating action of barium and the introduction of more sodium chloride into the muscle may favor this stimulation.

While working on the stimulation of a muscle by the outward diffusion of previously introduced barium salts, I had frequent occasion to observe the strong stimulation of the muscle when it was removed from the barium solution. In 1901 Loeb (8) discovered the curious phenomenon that a muscle may be perfectly quiescent in a certain solution but is thrown into violent twitches by the removal of the solution, these contractions ceasing almost immediately upon replacing the muscle in the original solution. The solutions which Loeb found capable of causing this reaction are, with one exception, the salts of sodium which cause precipitation or inactivation of calcium, such as the oxalate, fluoride, tartrate and citrate of sodium. This most unusual behavior of the muscle Loeb called contact reaction and the form of irritability developed in the muscle by the above-named reagents he designated as "contact irritability." Subsequently I (9) showed that it is not necessary to precipitate the calcium in the muscle in order to establish this contact irritability for the previous bathing of a muscle with a solution of the chloride of rubidium or caesium enables various other salts of sodium, lithium and ammonium to call forth contact reactions.

When the gastrocnemius muscle of a frog is placed in a solution composed of 10 cc. 0.2 m dextrose plus 3 drops of m/8 barium chloride, nothing may happen for one or two hours; but the moment the solution is removed, the muscle goes into contractions which cease when it is brought back into the solution. In figure 1 the muscle, previous to *a*, has been in the above named solution for thirty minutes; at *a* the solution is removed and as a result, the hitherto quiet muscle is thrown into a strong contraction; the replacing of the barium chloride and cane sugar solution at *b* quiets the muscle instantly. At *c* the removal of the solution once more causes activity on the part of the muscle. When the barium chloride is diluted with a 0.7 per cent sodium chloride solution instead of 6 or 7 per cent cane sugar, no contact reaction takes place. A muscle in 10 cc. m/8 NaCl plus 2 or 3 drops m/8 $BaCl_2$ shows, after a latent period varying from two to thirty minutes, energetic twitches as long as the muscle remains in the solution; on removing the muscle from the solution the twitches cease very speedily, to begin again on replacing the muscle in the solution.

The behavior of a muscle placed in a m/420 $BaCl_2$ when this is diluted with 7 per cent cane sugar is, therefore, very different from that of a muscle bathed with m/420 $BaCl_2$ in 0.7 per cent NaCl. In the first solution the muscle remains quiet for an almost indefinite period, but instantly goes into strong contraction when it is removed from the solution; in the second case the muscle is soon thrown into twitches of greater or lesser violence which cease very soon after the solution is removed. In other words, barium in the presence of sodium stimulates as long as it is in contact with the muscle while in the presence of sugar barium acts only on the removal of the muscle from the solution. The reason for this difference lies in the influence of sodium salts on the twofold action of barium. This twofold action of barium consists of: first, what I shall call the "direct" or ordinary stimulating effect when a muscle is immersed in a solution of a barium salt; second, aside from this usual stimulating action which barium shares with many other chemical compounds, barium calls forth, under the proper conditions, a contact reaction which is decreased or altogether inhibited by many of the sodium salts.

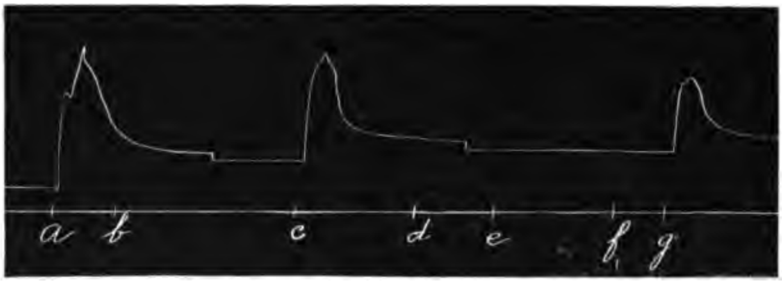

Fig. 1. At *a* and *c* the muscle is removed from a solution composed of 10 cc. 0.2 m dextrose + 3 drops m/8 $BaCl_2$. At *b* the solution is replaced. At *d* 3 drops of m/8 NaCl are added to the above solution; at *c* the muscle is removed. At *f* the original solution used at *a* is replaced and at *g* the muscle is removed from it.

This action of sodium in retarding the development of contact reaction by barium can be proved in various ways:

1. While m/420 barium chloride in 7 per cent cane sugar gives rise to contact reaction in ten or twenty minutes, this same concentration of barium in 0.7 per cent sodium chloride has no such effect.

2. In figure 1 the muscle bathed with 10 cc. 0.2 m cane sugar plus 3 drops m/8 $BaCl_2$ gives rise to contact reaction shown at *a* and *c*; at *d* the muscle is returned to the solution after three drops of m/8 sodium chloride have been added. Eleven minutes later, at *e*, the muscle is again exposed to the air but, it will be seen, no contact reaction takes place. At *f* the original solution of barium chloride in cane sugar is

replaced and, after seven minutes, removal of the muscle from the solution, at *g*, once more causes contact reaction.

3. The two gastrocnemius muscles of a frog were prepared; one was placed for twenty minutes in m/8 sodium chloride; the other, in 7 per cent cane sugar for the same length of time. At the expiration of this period, both muscles were immersed in 10 cc. 7 per cent cane sugar plus 3 drops $BaCl_2$; the muscle previously bathed with cane sugar showed contact reaction in fifteen minutes while the muscle treated with sodium chloride did not for thirty-five minutes. It is evident that the removal of sodium salts from the muscle previous to the application of barium favors the development of contact irritability.

4. This antagonism of sodium to barium can also be demonstrated in the following manner. The addition of 1 or 2 drops of sodium chloride to a solution of cane sugar and barium chloride in which the muscle has developed contact reaction very promptly abolishes the reaction; if now a few more drops of barium chloride are added, the muscle again acquires contact irritability.

5. If the sodium chloride is added immediately to the barium solution the usual contact reaction does not appear; thus, in 10 cc. 7 per cent cane sugar plus 3 drops m/8 $BaCl_2$ plus 5 drops m/8 NaCl the muscle did not develop contact irritability although it was allowed to remain in the solution for more than an hour.

What has been said concerning the antagonistic influence of the chloride of sodium, also applies to the bromide, iodide, nitrate, nitrite and chlorate; this is somewhat surprising for most of the above-named salts themselves stimulate the muscle-nerve preparation, being in this respect far superior to the sodium chloride. Mathews (10) gives the order of increasing power to stimulate the motor nerve as:

$$NaCl < NaBr < NaI.$$

I (11) found that while the gastrocnemius muscle may remain for two or three hours in a m/8 sodium chloride without any contractions appearing, in a m/8 sodium iodide or bromide solution the muscle is very soon thrown into violent twitches. Hence, we have here an example of an agent which in itself stimulates greatly but which inhibits the stimulation brought about by another agency. A few experiments with sodium acetate and citrate gave opposite results to those obtained with the iodide, bromide, etc., but this will be detailed in a future article. The chloride of potassium, calcium, lithium and magnesium all abolish the contact irritability established by barium salts. Also may it be stated that barium nitrate behaves in same manner as the chloride.

The production of contact irritability by a solution containing cane sugar and a barium salt is due primarily, I think, to the barium. A cane sugar solution having an osmotic pressure equivalent to that of the muscle—a 0.2286 mole, according to Webster (12)—does not cause contact reaction; even a 0.35 m cane sugar and a 0.8 m dextrose solution fail to do so, even though they are applied for one or two hours. Whether the sugar solution plays any other part than to serve as a diluent for the barium salt and as an indifferent fluid which allows the sodium salts to diffuse out of the muscle has not been definitely ascertained. Neither have I gained any further knowledge as to the external condition that causes a muscle to go into contraction on its removal from a solution in which it had remained at rest for an hour or more. This investigation concerns itself merely with the striking fact that barium has a twofold action on the muscle-nerve preparation; first, a direct stimulation and, second, the development of contact irritability. The first of these effects of barium is not only favored by sodium salts but absolutely depends upon the presence of these salts. This acceleration is especially true for such salts of sodium as the bromide and iodide which in themselves stimulate the muscle and which in the presence of barium add their effects to that of barium; they may therefore be regarded as synergistic agents. On the other hand, these same salts of sodium antagonize the action of barium in establishing contact reaction.

This peculiar behavior of sodium salts is not due to any action upon the motor nerve as the following experiments prove. The sciatic nerve of a muscle-nerve preparation was placed in a solution of 10 cc. m/8 NaCl plus 3 drops of m/8 $BaCl_2$ for one or two hours; judged by the condition of the muscle, this solution failed to stimulate the nerve. When the muscle is placed in this concentration of barium chloride, it goes into twitches in from two to ten minutes. During a brief stay in the dilute barium chloride the nerve is not injured, for its irritability, as measured by the strength of faradic current necessary to stimulate, is actually greater thirty or forty minutes after its immersion in the barium solution than before. However, this may be only an apparent increase in irritability, due, as Shōji (13) showed, to the increased electrical conductivity caused by the passing of the salt into the nerve. Neither is a nerve stimulated by m/420 $BaCl_2$ in a 0.2 m cane sugar or dextrose solution, nor is the removal of the nerve from this solution followed by any activity on the part of the muscle, i.e., no contact irritability is established. It is therefore highly probable that these two effects of barium salts are brought about in the muscle itself.

The explanation of these two effects of barium and the synergistic action of sodium salts in the usual form of stimulation by barium and their antagonistic action in the production of contact reaction, no doubt, depends upon the explanation that must still be found for the contact phenomenon in general. Meanwhile two explanations suggest themselves. It is conceivable that barium in the presence of sodium stimulates a certain structure in the muscle and thereby causes the muscle to exhibit the usual contractions observed as long as the muscle remains in the solution. But, in the absence of sodium, barium merely so alters this structure that it readily responds to a new (or additional) form of stimulus, the nature of which is at present unknown and which becomes operative only when the muscle is removed from the solution; the change brought about by barium in this irritable structure is prevented by sodium salts. Or we may presuppose two irritable structures in the muscle, e.g., the muscle fiber itself and the receptive substance. One of these is acted upon synergistically by sodium and barium; this leads to what we have called the direct stimulation. The other structure is so affected by the barium that contact irritability is established and the establishing of this contact irritability is prevented by sodium. Both these actions of barium are antagonized by potassium, calcium and magnesium ions.

SUMMARY

1. Barium salts have two effects upon the muscle:

a, They cause "direct stimulation" which results in contractions of the muscle as long as the muscle remains in the solution.

b, They establish "contact irritability" which the muscle exhibits only when it is removed from the solution.

2. For the "direct stimulation" by barium the presence of sodium salts is necessary; removal of these salts by immersing the muscle in an isotonic cane sugar or dextrose solution renders this stimulation impossible.

3. In the establishing of "contact irritability" by barium, certain sodium salts act antagonistically and therefore their removal by bathing the muscle in a sugar solution is necessary.

4. Both the direct stimulation and the contact reaction are antagonized by potassium, calcium and magnesium.

BIBLIOGRAPHY

(1) RINGER: Journ. Physiol., 1886, vii, 291.
(2) LINGLE: This Journal, 1900, iv, 265.
(3) LINGLE: This Journal, 1902, viii, 75.
(4) LOEB: Arch. f. d. gesammt. Physiol., 1900, lxxx, 229.
(5) OVERTON: Arch. f. d. gesammt. Physiol., 1904, cv, 176.
(6) ZOETHOUT: This Journal, 1919, xlvii, 161.
(7) LILLIE: This Journal, 1902, vii, 25.
(8) LOEB: This Journal, 1901, v, 362.
(9) ZOETHOUT: This Journal, 1904, x, 324.
(10) MATHEWS: Science, N. S., 1902, xv, 492.
(11) ZOETHOUT: This Journal, 1904, x, 211.
(12) WEBSTER: The Decennial Publications, Univ. of Chicago, 1902, x, 105.
(13) SHŌJI: This Journal, 1919, xlvii, 523.

DISTRIBUTION OF THE ANTINEURITIC VITAMINE IN THE WHEAT AND CORN KERNEL

A CONTRIBUTION TO THE BIOLOGY OF THE ANTINEURITIC VITAMINE

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The work of the last ten years on the presence of the antineuritic vitamine in foodstuffs has conclusively demonstrated that the seeds of the ordinary cereals are relatively rich in this accessory food. The study of beriberi, for instance, has shown that the whole rice kernel is a prophylactic against this disease, whereas highly milled rice is devoid of this property. The difference in the two kinds of rice is, that the highly milled variety does not contain the peripheral layers nor the germ of the seed, these portions being removed with the "offal" in the milling process. It was found, furthermore, that the rice offal (polishings) contain the antineuritic vitamine, and the question then arose as to the location of the substance in the peripheral layers of rice. Funk (1) and many others claimed that the seat of the vitamine was to be found in the aleurone cells. This claim was, however, never substantiated by experimental evidence, but it nevertheless led to a similar doctrine regarding the distribution of the antineuritic vitamine in the wheat and corn kernel.

In 1915 McCollum and Davis (2) were able to show that the wheat embryo is more efficient in promoting growth than the entire wheat. It should be stated that these authors used as wheat germ a product which was obtained from a flour mill and which, although being largely composed of the embryo of wheat, was contaminated by other portions of the grain. About the same time Voegtlin, Sullivan and Myers (3) demonstrated that commercial corn germ has prophylactic value in avian polyneuritis.

A little later, extensive investigations by Voegtlin and Myers (4) on the relative food value of various wheat flours and corn foods, brought out the fact that those of the modern mill products which were particularly rich in embryo, of either corn or wheat, were also very effective in the treatment and prevention of the polyneuritis of pigeons.

During the progress of the present investigation an article appeared by Chick and Hume (5) in which these authors also call attention to the germ of cereals as being the chief seat of the antineuritic substance. They consider the aleurone layer as next in importance in this respect.

From the foregoing, it is evident that the embryo of corn and wheat is probably the part of the seed which contains a considerable amount of antineuritic vitamine. This conclusion is fairly well justified although, as has already been pointed out, the ordinary commercial wheat or corn germ does not contain the histological elements of the embryo only, but also some bran and endosperm. For theoretical and practical reasons it was very important to follow out this line of investigation with a view to decide the following questions:

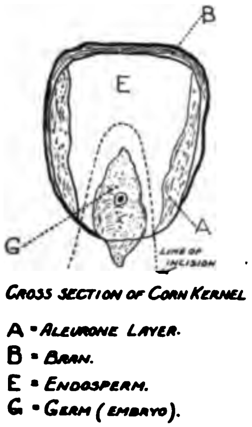


Fig. 1

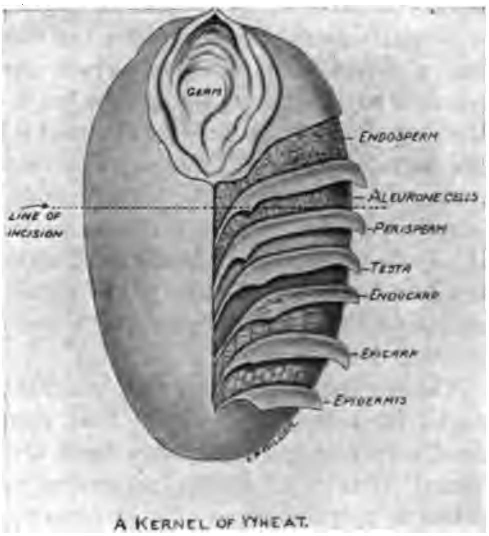


Fig. 2

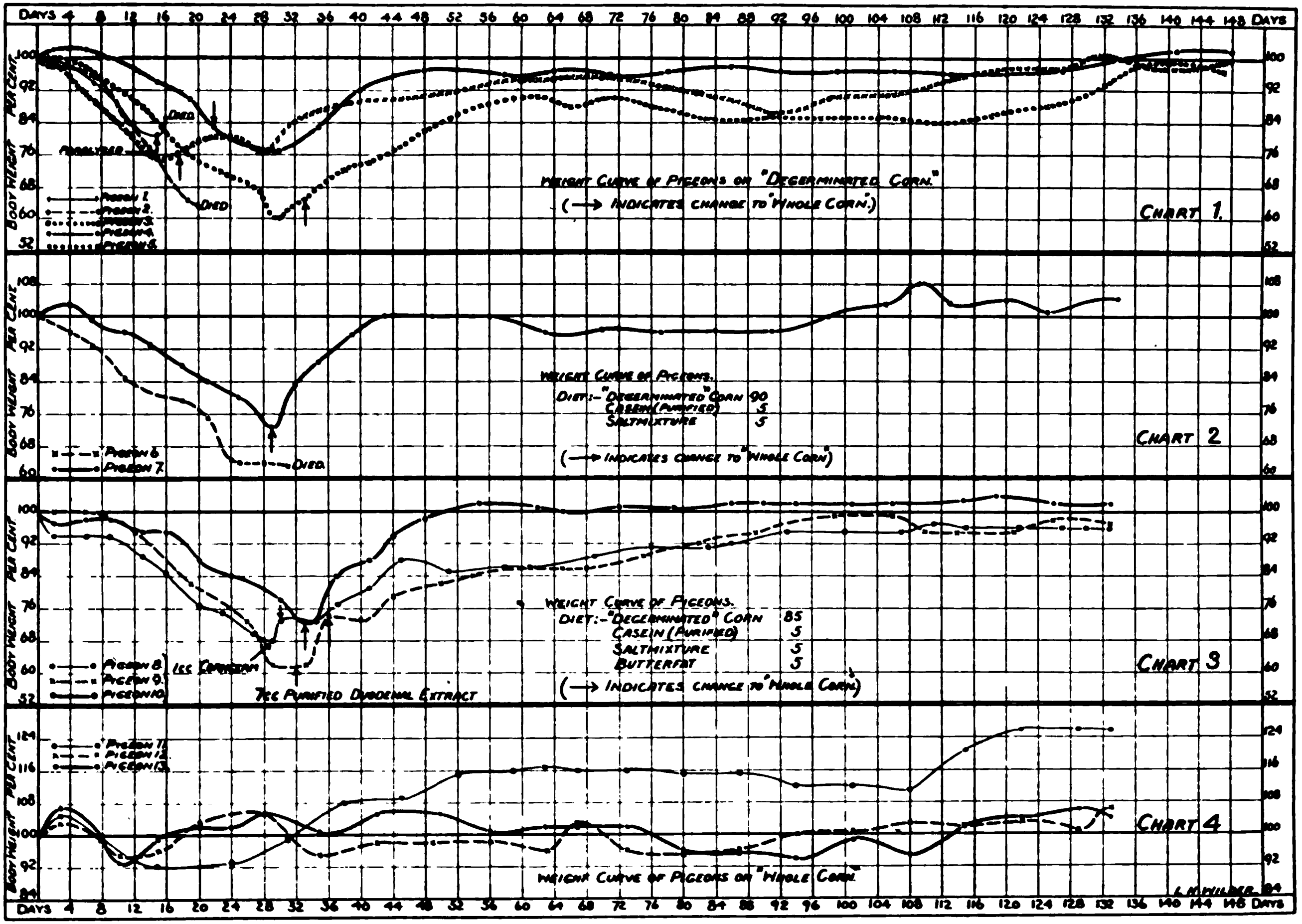
Is the antineuritic vitamine located in both the aleurone layer and the embryo, or only in one of these tissue elements?

The relations of the various parts of the corn and wheat kernel are illustrated by figures 1 and 2. The research was carried out on adult pigeons, series of which were fed on the following mixtures:

1. "Degerminated" corn, corn kernel from which the germ had been removed.
2. "Degerminated" corn, 90 per cent, casein (purified), 5 per cent, salt mixture, 5 per cent.
3. "Degerminated" corn, 85 per cent, casein (purified), 5 per cent, salt mixture, 5 per cent, butter fat, 5 per cent.
4. Whole corn.
5. "Degerminated" wheat.

In regard to the removal of the germ, it should be emphasized that this has to be carried out with painstaking accuracy, as otherwise the results of the feeding experiments are misleading. In the case of corn it is relatively easy to separate all of the germ from the endosperm by means of two sharp incisions, as indicated in figure 1. The corn endosperm is hard, whereas the germ is much softer, a fact which facilitates their separation. In the case of wheat it is necessary to sacrifice nearly one-half of the kernel by means of a cross-section (fig. 2). The wheat germ adheres very firmly to the endosperm and is very difficult to remove completely. As a matter of fact, we are convinced that in the early part of the experiment, illustrated by chart 5, not all of the germ had been completely removed, which explains the delay in the onset of polyneuritis. Soon after this defect was remedied the pigeons developed the disease.

The results obtained are very conclusive and need very little discussion. It is perfectly evident that removal of the germ or embryo from corn and wheat deprives these cereals of their antineuritic vitamine. That the disease appearing on a diet of "degerminated" cereal is polyneuritis is evident from *a*, the typical symptoms; *b*, the histological changes in the sciatic nerve; and *c*, the fact that the administration of an alcoholic extract of corn germ or autolyzed brewer's yeast leads to a prompt disappearance of the symptoms. It is, furthermore, interesting to note the rapid recovery of body weight and well-being following a change to whole corn or wheat, respectively. Proof that the symptoms are not due to the deficiency of the "degerminated" cereal in other essential foods is furnished by the experiments illustrated by charts 2 and 3. Chart 4 and other unpublished data show that pigeons can live in perfect health for a period of at least four months on an exclusive diet of either the whole corn or wheat kernel.


DAYS 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100 104 108 112 116 120 124 128 132 136 140 144 148 DAYS
BODY WEIGHT PER CENT
100 92 84 76 68 60 52
Paralysed
Died
Died
Pigeon 1.
Pigeon 2.
Pigeon 3.
Pigeon 4.
Pigeon 5.
Weight Curve of Pigeons on "Degerminated Corn."
(→ Indicates change to "Whole Corn.")
Chart 1.
108 100 92 84 76 68 60
Pigeon 6
Pigeon 7.
Died
Weight Curve of Pigeons.
Diet:- "Degerminated" Corn 90
Casein (Purified) 5
Saltmixture 5
(→ Indicates change to "Whole Corn")
Chart 2
Pigeon 8.
Pigeon 9.
Pigeon 10.
1cc Coprosan
7cc Purified Duodenal Extract
Weight Curve of Pigeons.
Diet:- "Degerminated" Corn 85
Casein (Purified) 5
Saltmixture 5
Butterfat 5
(→ Indicates change to "Whole Corn")
Chart 3
124 116 108 100 92 84
Pigeon 11.
Pigeon 12.
Pigeon 13.
Weight Curve of Pigeons on "Whole Corn"
Chart 4
L. H. Wilder

Charts 1–4

COMMENTS ON RESULTS

It would appear from the results obtained in this research that the antineuritic substance of corn and wheat is located entirely in the embryo of the grain. This discovery is of considerable interest: *a*, in relation to the biological significance of the antineuritic vitamine, and *b*, on account of its bearing on practical dietetics.

During the process of germination the metabolism is particularly active in the embryo, the necessary energy being furnished by the deposit of starch and protein in the endosperm. The fact that the vitamine is located in the germ and not in the endosperm might be considered as evidence that the growth of the young plant (cell division and differentiation) is dependent on the presence of this particular vitamine. There are no known facts which would contradict such an assumption. On the contrary it is very well established that the growth of animals is also dependent on the presence of a minimum amount of antineuritic substance. The following objection might possibly be raised to this view: Animals have to depend ultimately on plants for their supply of antineuritic substance, as the organs of animals are incapable of producing the substance from other materials, while *plants can synthetize the antineuritic vitamine.* Taking this into consideration, however, it is probably more than a mere coincidence that the corn and wheat embryo should contain the entire vitamine supply of the kernel. It is very likely that the presence of this substance is needed in the metabolic processes associated with the formation of the elements of the young plant. What these particular processes are is difficult to say, although the antineuritic vitamine is

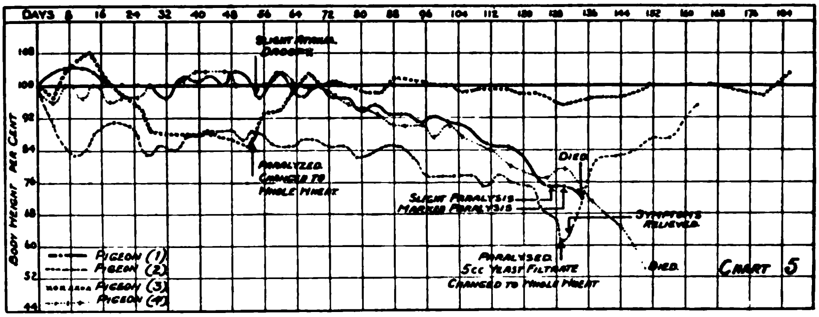


Chart 5

perhaps concerned in some way or another with the oxidative functions (see Dutcher and Collatz, 16).

That the antineuritic substance has some relation to metabolism is indicated by various other observations. One of these is the distribution of the substance in the organs of the animal body. From the work of Cooper (7), Osborne and Mendel (8) and others, it would appear that organs with a high rate of metabolism are also rich in antineuritic vitamine. The liver and the heart muscle, for instance, are very rich in vitamine and are also known to be places of especially high metabolic activity. Skeletal muscle, pancreas, kidney, brain, etc., come next in line. It is, furthermore, significant that the first symptoms in so-called "wet" beriberi point to the heart as being primarily affected by the vitamine deficiency of the diet. It is also striking that egg yolk and beans should be rich in vitamine; whereas egg white, the green parts of plants[1] and fruits (such as oranges) are relatively poor in this substance. The yeast cell, with its extraordinarily active metabolism, is also extremely rich in vitamine.

These considerations are summarized in table 1 compiled from the observations of various authors.

To return to the practical aspects of the present research, it might be well to call attention to the bearing of these results on the question of the nutritive value of bread. It is evident that as long as it was assumed that the antineuritic vitamine was located principally in the aleurone cells, it was not so objectionable to remove the germ during the milling process. Our present knowledge, however, would indicate, that by excluding the germ from the flour and cornmeal we deprive these foods of some of their most valuable dietary properties.[2] It should be possible so to alter the milling process as to allow the germ of the cereal to be incorporated in the finished product. A flour or cornmeal containing the germ would, of course, yield bread of a slightly darker color. On the other hand, it is taken for granted that flour or corn meal containing the embryo could not be stored for as long a time as the ordinary highly-milled products, without undergoing some deterioration. This is a considerable disadvantage, but could be partly overcome by proper harvesting and storage.

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[1] Since this article went to press a paper by Osborne and Mendel (J. Biol. Chem. 1919, xxxvii, 187) appeared, in which these authors state that dried spinach leaves contain about one-fourth as much antineuritic vitamine as brewer's yeast. The green parts of plants are evidently not as poor in this vitamine as has been assumed hitherto. This observation substantiates our conception of the relation between metabolic activity and antineuritic vitamine, as the metabolism of the leaves of vegetables is certainly very active during the growth of the plant.

[2] The germ also contains a considerable amount of fat soluble vitamine.

A considerable number of investigators have conceded the higher nutritive value of war bread made from "low extraction" flour (containing the germ and some of the bran). The main objection to this flour is attributed to its excessive cellulose content, which may lead to intestinal disturbances in susceptible individuals. We believe that a product which does not contain any bran but does include the germ, would not possess these objectionable features, would at the same time be more nutritious and would reduce greatly the possibility of vitamine deficiency in the modern mixed diet. Increasing the vitamine supply in our diet should be our aim in order to insure a sufficient margin of safety, and increasing the vitamine content in our daily bread would go a long way toward accomplishing this end.

TABLE 1

FOOD STUFF	DAILY RATION REQUIRED TO PREVENT POLYNEURITIS (IN TERMS OF DRY WEIGHT)	AUTHORS
	grams	
Yeast (pressed)	0.5	Cooper
Ox liver	0.9	Cooper
Wheat germ	1.3	Chick and Hume
Egg yolk	1.5	Cooper
Ox heart	2.0	Cooper
Fish (hake)	More than 2.0	Cooper
Sheep brain	2.5	Cooper
Lentils	3.0	Cooper
Barley	4.5	Cooper
Beef	5.0	Cooper
Cheese	More than 5.6	Chick and Hume
Cow's milk	More than 3.5	Chick and Hume

SUMMARY

1. The germ or embryo of the wheat and corn kernel contains all of the antineuritic vitamine of these cereals.

2. Wheat flour or corn meal containing the germ is, therefore, more nutritious than the corresponding highly-milled products.

3. Consideration of the distribution of the antineuritic substance in the wheat and corn kernel and in the animal body suggests that this

accessory food is necessary for the metabolism of the growing plant as well as the animal body. It appears that cells with an especially active metabolism are also rich in antineuritic vitamine.

BIBLIOGRAPHY

(1) **Funk**: Die Vitamine, Wiesbaden, 1914.
(2) **McCollum and Davis**: Journ: Biol. Chem., 1915, xxi, 179.
(3) **Voegtlin, Sullivan and Myers**: Public Health Rept. 1916, August 14.
(4) **Voegtlin and Myers**: Public Health Rept., 1918, May 3, May 31 and June 7.
(5) **Chick and Hume**: Journ. Roy. Army Med. Corps, 1917, xxix, 121.
(6) **Dutcher and Collatz**: Journ. Biol. Chem., 1918, xxxvi, 63; 1918, xxxvi, 547.
(7) **Cooper**: Journ. Hyg., 1912, xii, 436.
(8) **Osborne and Mendel**: Journ. Biol. Chem., 1917, xxxii, 309; 1918, xxxiv, 17.

PRESSURE CHANGES IN THE CEREBRO-SPINAL FLUID FOLLOWING INTRAVENOUS INJECTION OF SOLUTIONS OF VARIOUS CONCENTRATIONS

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In an attempt to determine whether, following intravenous injection of hypertonic solutions of sodium chloride, an increased amount of this salt could be detected in the cerebro-spinal fluid, it was noted that within a short time after the intravenous injection, cerebro-spinal fluid could not be obtained when the subarachnoid space was entered. This observation led at once to the attachment of a manometer to the puncture needle for the purpose of making continuous readings of the cerebro-spinal fluid pressure following the intravenous injection of hypertonic solutions of sodium chloride. It was soon found that the pressure of the cerebro-spinal fluid could be altered very rapidly and very definitely by intravenous injections of solutions of various concentrations.

METHOD

Cats were used entirely in these experiments and the subarachnoid space was entered by puncture with a needle through the occipito-atlantoid ligament. All animals were placed, lying on their sides, on a level board and kept thus throughout the pressure observations. As soon as a puncture was made a glass manometer was connected with the puncture needle by means of a metal elbow. For observations where previous experience made it certain that the pressure would remain above zero, a straight manometer was used, but for observations involving pressures below zero a U-shaped manometer, filled to zero with Ringer's solution, was connected with the puncture needle. The manometer was held in the vertical position throughout the experiment by a burette clamp. As soon as the manometer was attached, direct readings were begun and continued every half-minute for eighty or more minutes. Each reading was recorded as made and curves plotted at the end of each experiment. Our experience has shown that any manipulation of the puncture needle and of the manometer should be avoided if possible. After a good entry of the subarachnoid space uniform conditions are thus assured throughout the period of the experimentation. Sixteen to eighteen gauge puncture needles and manometers with a bore of 1 to 1.5 mm. were used in these observations.

Ether was chosen as the anesthetic largely because experience showed that the concentration of the anesthetic had to be regulated in accordance with the solution injected. It was necessary in many cases to diminish the amount administered for some minutes to avoid death of the animal, particularly when the initial injection was given too rapidly. Although with a non-volatile anesthetic a more even narcosis might be assumed, the disadvantages of this method were found to outweigh its advantages. In the early experiments ether was given by cone but in later observations a very even and satisfactory anesthesia was carried out by the well-known method of intratracheal insufflation. In many of the routine experiments a constant adjustment of the air and ether volume by this method was maintained throughout.

Variation in the temperature of the animals during these long observations under ether was found to affect the general condition of the animal and consequently the pressure of the cerebro-spinal fluid, so that an attempt was made to maintain the body temperature of the animals during the experiments by means of suitably adjusted electric lamps.

Injections of the solutions of different concentrations were all made intravenously. In the early experiments the injections were made with a syringe but owing to the difficulty of control the later injections were all given from a burette connected by rubber tubing with a venous cannula.

NORMAL PRESSURES

A number of readings of the normal pressure of the cerebro-spinal fluid were made in cats under ether anesthesia. At first these readings were isolated observations and as soon as the fluid in the manometer reached a fairly constant level, the record was made. Such observations were made on twenty cats, under routine experimental conditions and an average pressure of 119 mm. of cerebro-spinal fluid was obtained. The maximal recorded pressure was 155 mm. and the minimal 90 mm.

But these observations were all made on animals which were immediately thereafter used for other purposes. The method of recording the pressure was imperfect in that a considerable dislocation of the cerebro-spinal fluid occurred; this dislocated fluid was required to fill the manometer to the initial level. Likewise the ether was administered by cone, a method which gave varying concentrations of the anesthetic. It was felt that the true pressure of the fluid could only be obtained after sufficient time had elapsed to permit any compensation for the dislocated fluid. In addition, in connection of the manometer to the needle, some fluid was very frequently lost; this loss gave a lower initial reading and necessitated further replacement. Hence experiments were undertaken, with the anesthetic administered by the intratracheal method, extending over a sufficient period of time to permit all physiological compensation for displacement and loss of fluid. For the most part such compensation was accomplished within the first fifteen minutes, as shown by the subsequent records.

In our observations the excursions of the column of cerebro-spinal fluid in the manometer, due to inspiration and expiration, were influenced by the method of anesthesia. With the administration of ether through the intratracheal tube the character and extent of the respiratory movements are altered. With an intratracheal tube of the proper size, sufficient space is left in the trachea and larynx to allow a free return to the exterior. Asphyxia seems to be wholly avoided by the proper regulation of the intratracheal blast. The respiratory movements may be controlled by the volume of the blast; in these experiments it was planned to maintain sufficient movement in inspiration and expiration to give to the column of fluid in the manometer connected with the subarachnoid space, well-marked excursions. Under these conditions these excursions varied from 1 mm. to 6 mm., usually being about 4 mm. in amplitude. These excursions were measured from the low point reached in inspiration to the high point reached in expiration. Pressure on the thorax in our experiments caused a rise of several centimeters in the fluid column in the manometer. Along with the respiratory excursions just noted, there also occur in the fluid column, pulsations due to the heart-beat. In our experiments these pulsations varied from 0.5 mm. to 2 mm. under certain conditions. The usual amplitude of these cardiac pulsations was 1 mm.

Under experimental conditions assuring constant anesthesia and constant body temperature, a number of continuous readings of the pressure of the cerebro-spinal fluid were made over a period of eighty minutes. Figure 1 is a composite constructed from seven such observations and shows the maintenance of a very uniform pressure throughout, though with a slight rise in the readings during the first fifty minutes.

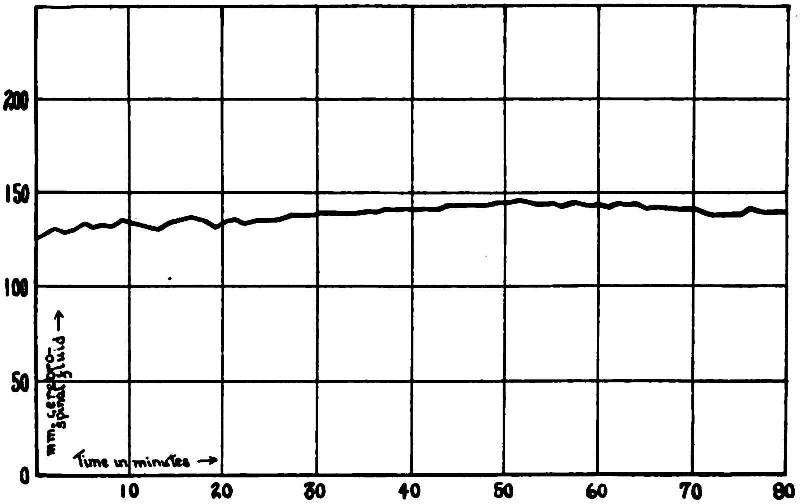

Fig. 1. Composite curve showing normal pressure of cerebro-spinal fluid. Composite made from seven individual curves.

Figure 2 shows a curve of normal pressure of a single cat. It will be noted that in the first five minutes of the observation there is a considerable rise in the pressure. This is quite characteristic in many of our observations and may be accounted for by two factors. First, following the release of the cerebro-spinal fluid by the puncture, dislocation of some of the fluid to fill the manometer was inevitable; second, in the connection of the manometer with the puncture needle the loss of at least one drop of cerebro-spinal fluid occurred in this observation. This initial rise then probably represents, partially at least, the restoration of the fluid displaced and lost in the connection of the manometer. The rise in the curve is unusually abrupt and the compensation seems complete as indicated by the maintenance for the remainder of the experiment (70 minutes) of a uniform pressure. During this observation, when the pressure readings were constantly at the same level, the respiratory excursions in the column of fluid in the manometer were 3 mm. and the arterial variations 0.5 to 2 mm. The uniformity of the pressure readings indicates that under the experimental conditions prevailing, reliable data were obtained.

In figure 1, the composite record of seven observations, it is indicated that the pressure of the cerebro-spinal fluid in the etherized cat was from 135 to 145 mm., with the rise occurring largely in the early part of the curve. Comparable values for the pressure of the cerebro-spinal fluid were obtained from many other cats, both as initial readings and as values of the pressure after the completion of the initial rise. In this series of sixty-five cats, which includes the twenty already mentioned, the initial pressure averaged 119 mm. of cerebro-spinal fluid; the subsequent pressure (after early compensation had occurred), as based on twenty-one animals, averaged 129 mm. of cerebro-spinal fluid. The interesting relationship of the lower value of the initial reading to the later more constant pressure is thereby indicated.

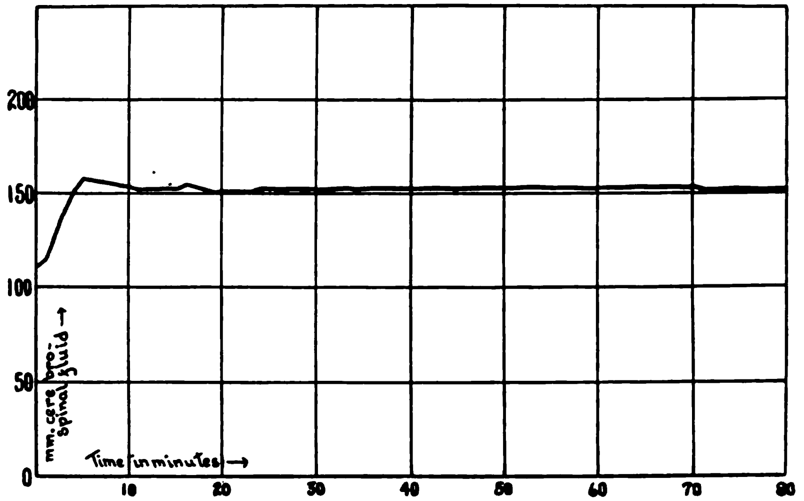

Fig. 2. Cat no. 1319. Normal pressure of cerebro-spinal fluid

Analysis of the initial pressures of the cerebro-spinal fluid in these sixty-five cases shows that in only eight cats was the reading less than 100 mm. In several of these a loss of three or more drops of fluid, in connecting the manometer, was noted; one animal was obviously in poor physical condition at the time of experimentation. Others of these eight cats had been subjected to previous experimental procedures in the laboratory. Thirty-six of the sixty-five cats gave initial readings between 100 and 120 mm. with an average of 110 mm., while seven cats fell between 120 and 140 mm. with an average of 131 mm. Fourteen cats showed an initial pressure of over 140 mm. with an average of 153 mm. Five of the fourteen had been previously used for experiments leading to the possible production of a chronic meningitis. In none of these fourteen was the fluid loss in the connection of the manometer more than one drop. Further, the cats which had not been subjected to previous experimentation gave the lower values in this series of fourteen.

The increased elaboration of the cerebro-spinal fluid under the influence of the volatile anesthetics (ether, chloroform) has been noted many times since Cappelletti (1) first described this phenomenon. Pettit and Girard (2), and later Dandy and Blackfan (3) and Dixon and Halliburton (4) have emphasized its importance. All the observations have dealt with an increased production of fluid as determined by the rate of flow from a needle or cannula in the subarachnoid space, the pressure of the cerebro-spinal fluid being determined by the capillary resistance to flow in the cannula. Although no attempt has been made in these experiments to study extensively the effects of variations in the concentration of the anesthetic on the pressure of the cerebro-spinal fluid, some data relating to the pressure of the fluid under these conditions have been obtained.

In our earlier observations, where ether was given by cone, it soon became apparent that variations in the concentration of the ether were sufficient to cause changes of appreciable magnitude in the pressure of the cerebro-spinal fluid as read in the manometer. Analysis of such experiments where there were changes in the pressure of the cerebro-spinal fluid due to variations in the ether supply, shows that the pressure will vary from 5 to 20 mm. with slight changes in the anesthetic during its routine administration by cone. Other, greater changes in the pressure have occurred in animals which were brought rather quickly from very light to full anesthesia or vice versa. Associated with the possible increase in the production of cerebro-spinal fluid due to the ether are other equally significant vascular readjustments; the combination of the two factors within the cranium determines the resulting pressure change in the spinal fluid. Similarly, exhaustion of the secreting cells of the choroid plexus by the anesthesia may present an additional element which in the latter parts of the observation causes further alterations in the fluid pressure.

Such considerations lead to the view that the pressures recorded as normal in foregoing paragraphs are only relatively and not absolutely accurate. For the values are influenced by the displacement of fluid in the manometer, by loss of fluid in connecting the apparatus and by the anesthesia. The alterations in pressure referable to the anesthetic and associated asphyxia have been controlled throughout so that these factors are constant in all of our observations. Partial or complete compensation for dislocation or loss of fluid is quite uniform and occurs largely during the first few minutes of experimentation. Such relatively constant records of the pressure of the spinal fluid give a reasonable basis upon which further experimental data may be interpreted.

RINGER'S SOLUTION

Following the above observations in which the normal pressure has been plotted over periods of eighty minutes, the effects of various quantities of Ringer's solution, injected intravenously, were considered. The formula for the Ringer's solution used is as follows: NaCl, 0.9 per cent; KCl, 0.042 per cent; $CaCl_2$, 0.025 per cent. In the experiments in which a relatively small amount of the solution (12 to 20 cc.) was injected, the curve showing the pressure of the cerebro-spinal fluid rises during the injection but falls on its completion to the level prevailing at the beginning of the experiment. This pressure is then maintained during the remainder of the observation. Such curves simulate very closely the normal except for the temporary rise during the injection. This initial rise is most marked when the intravenous injection is given very rapidly and with considerable pressure. It seemed apparent then that after the injection of small amounts of Ringer's solution physiological readjustment of the volume of the blood took place very rapidly so that the pressure of the cerebro-spinal fluid was not essentially affected.

Other experiments were performed in which a large quantity (100 cc.) of Ringer's solution was introduced intravenously in order to furnish control for observations (to be described later) in which 100 cc. of water were injected, and to determine if possible the effect on the pressure of the cerebro-spinal fluid of the intravenous introduction of this enormous volume of a solution isotonic with the blood. Figure 3 shows a record of the pressure of the cerebro-spinal fluid in such an experiment. In this case the injection was made from a burette connected with a cannula in a fore-leg vein and lasted for twenty-six minutes, being given then at a rate of 3.85 cc. per minute. During the injection of the first 33 cc. the rate was rapid (6.6 cc. per minute); and for the next 17 cc. the rate was 4.25 cc. per minute. Reference to figure 3, in which the rapidity of the injection is indicated on the base line showing the period of injection, reveals the fact that when the rate of introduction of the fluid was high, at the beginning of the injection, the pressure of the cerebro-spinal fluid rose more abruptly and more rapidly than when the rate was decreased. The mechanical effect of a difference in the rate of intravenous injection, on the pressure of the cerebro-spinal fluid, is thus indicated. A further matter of interest brought out by our observation during the injection period in this experiment, is that the impact of the injection seems to have an effect on the pressure of the cerebro-spinal fluid which is independent of the effect produced by rapid injection. This has been noted in many of our experiments. During the latter part of the period of injection the rate was low and it can be seen from figure 3 that the pressure of the cerebro-spinal fluid had already begun to fall. This decrease in pressure continued gradually and uniformly during the remainder of the observation, the fall gradually becoming less rapid. Within thirty minutes after the completion of the injection the pressure had dropped to 138 mm., a point probably well within the normal pressure variation in this animal. During the last thirty minutes of the experiment the pressure, which was falling very gradually, was practically the same as that noted before the injection of the Ringer's solution.

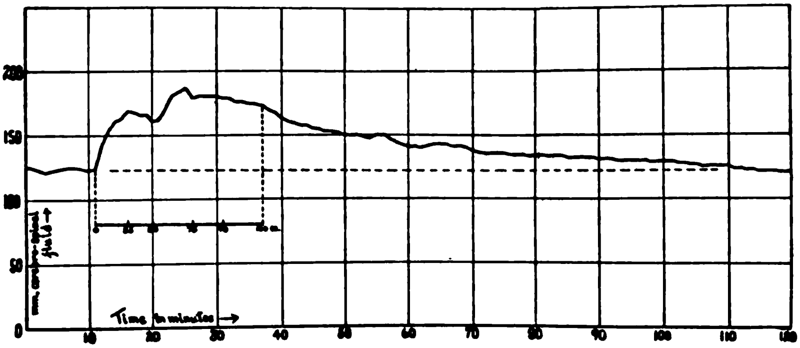

Fig. 3. Cat no. 1543. Pressure cerebro-spinal fluid with intravenous injection of 100 cc. of Ringer's solution.

It is apparent from the foregoing observation that the intravenous injection of a relatively large volume of fluid isotonic with the blood, although it increases the pressure of the cerebro-spinal fluid during the period of injection, does not bring about any material and lasting change in this pressure. The physiological adjustment to such a large volume is, however, a process requiring more time than is necessitated by the intravenous injection of smaller volumes. No ill-effects of this procedure are apparent in animals allowed to recover from the anesthetic.

HYPOTONIC SOLUTIONS

With the establishment of a curve of normal pressure of the cerebro-spinal fluid under the experimental conditions adopted, intravenous injections of distilled water were given. A prompt and enduring rise in the pressure of the cerebro-spinal fluid was recorded in every instance, even after the introduction of relatively small volumes. The animals showed no respiratory or cardiac difficulties during or after the injection of this hypotonic solution; recovery from ether at the end of the experiment was prompt and no after-effects were observed.

Such a marked and enduring rise in the pressure of the cerebro-spinal fluid is shown in figure 4, following an intravenous injection of 20 cc. of distilled water. An abrupt rise in pressure during the period of injection is recorded; as soon as the injection was stopped a slight fall occurred, giving an obtuse peak in the curve. Within five minutes after the end of the injection the pressure of the fluid had again started to rise and this upward tendency was maintained throughout the period of observation.

An increase in the pressure of the cerebro-spinal fluid is the typical response to the intravenous injection of a hypotonic solution (distilled water). The initial peak in the curve during the injection seems to be partially at least a mechanical effect due to the rapid introduction of fluid into the venous system. It may be lessened or avoided by the very slow injection of the foreign solution, indicating that the resultant persisting change in the pressure of the cerebro-spinal fluid is not an immediate phenomenon but requires a varying interval of time for its fulfilment. This interpretation is supported by the curves in figures 4 and 5; in both of these animals, especially in the one receiving the smaller dose, the effect of the hypotonic solution is continued and is at its maximum at the end of the observation. That in the intravenous injection of even a large amount of distilled water, the initial peak in the pressure curve may be avoided by giving the injection uniformly and gradually over a considerable period of time (26 minutes), is shown by figure 5. This curve may be compared

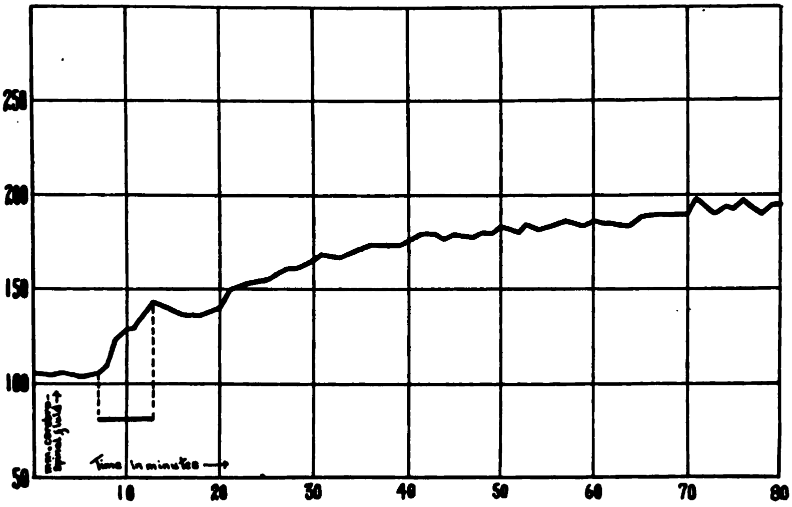

Fig. 4. Cat no. 1303. Pressure cerebro-spinal fluid with intravenous injection of 20 cc. sterile distilled water.

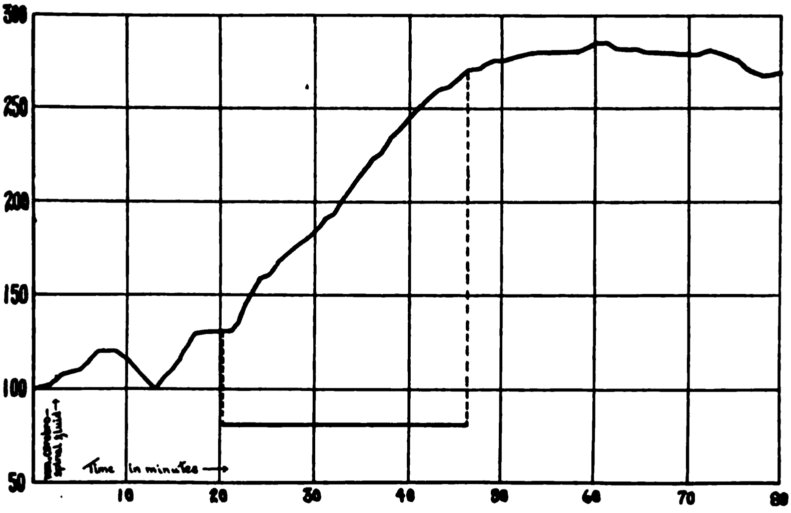

Fig. 5. Cat no. 1498. Pressure cerebro-spinal fluid with intravenous injection of 100 cc. sterile distilled water.

directly with figure 3, which represents the pressure of the cerebro-spinal fluid after the intravenous injection of a similar amount of Ringer's solution. In figure 5 the rise in pressure begins abruptly, is maintained throughout the period of introduction of fluid and continues even after its completion. In this experiment the long period of injection may have sufficed to include within it the time necessary for the readjustment of pressure relations, so that any fall after the initial injection was avoided. In the early part of the curve, before the injection of water, is a fall due to ether; but the pressure was constant at 130 mm. before the injection was begun. In this experiment, as seen from figure 5, there is recorded a rise in the pressure of the cerebro-spinal fluid from 130 to 285 mm. of this fluid.

These observations show definitely that the intravenous injection of a hypotonic solution (distilled water) causes a prompt and enduring rise in the pressure of the cerebro-spinal fluid. The animals, subjected to such injections and allowed to recover from the ether, promptly become normal.

HYPERTONIC SOLUTIONS

Many experiments dealing with the effect of the intravenous injection of concentrated solutions of the common sodium salts (chloride, bicarbonate and sulphate) upon the pressure of the cerebro-spinal fluid have been carried on. In general, this procedure results in a temporary rise in the pressure of the fluid followed by a marked and enduring fall.

Sodium chloride. With sodium chloride in 30 per cent concentration, striking results have been obtained, with continuous readings of the pressure of the cerebro-spinal fluid during a period of eighty minutes. All curves plotted from data arising from these experiments show an abrupt initial rise in the pressure of the fluid, usually from 50 to 75 mm. This continues throughout the period of intravenous injection though it frequently forms a plateau in the curve in the latter part of the process. As soon as the injection is completed, the pressure of the cerebro-spinal fluid starts to fall and a rapid lowering is recorded during the next few minutes. The rate of fall then decreases shortly before the pressure reaches its lowest point. The maximum effect of the intravenous injection, i.e., the point of lowest pressure, is usually noted in from fifteen to twenty minutes after the completion of the injection.

Figure 6 shows the effect of a single intravenous injection of 12 cc. of a 30 per cent solution of sodium chloride upon the pressure of the cerebro-spinal fluid. An initial rise of 80 mm. during the period of injection is recorded; following this a rapid and extreme lowering of the pressure occurred, the rate of fall decreasing only when the pressure went to below zero. The minimal pressure of the cerebro-spinal fluid recorded was minus 22 mm., a point reached eighteen minutes after the completion of the injection. The pressure then remained below zero for a period of twenty-six minutes. Subsequently it rose slowly to a pressure of 40 mm. above zero, which was reached at the end of the observation.

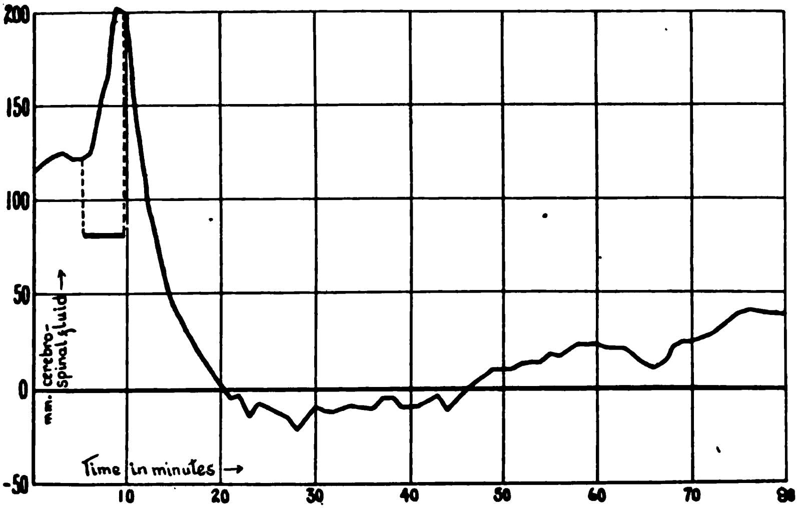

Fig. 6. Cat no. 1271. Pressure cerebro-spinal fluid with intravenous injection of 12 cc. 30 per cent sodium chloride.

It must not be assumed that the intravenous injection of concentrated solutions of sodium chloride inevitably brings about a lowering of the pressure of cerebro-spinal fluid to below zero. With various intravenous doses of this salt, roughly half of our experiments gave readings below zero. The maximum effect was obtained in one cat by repeated intravenous injections (total 22 cc.) of 30 per cent sodium chloride, when the pressure of the cerebro-spinal fluid was recorded as minus 55 mm.

In many other observations, the result of such injections of sodium chloride was a marked lowering of the pressure of the cerebro-spinal fluid (50 mm.) and the maintenance of this lowered pressure throughout the period of record. This seems to be the phenomenon of chief physiological importance—a reduction of the pressure to a lower level. A curve showing the result of an experiment of this nature is given in figure 7. In this case the pressure dropped abruptly for a period of ten minutes after the intravenous injection; this lowered pressure was maintained with minimal change for at least forty minutes.

Sodium bicarbonate. Of the six cats given intravenous injections of saturated solutions of sodium bicarbonate (about 18 per cent), only one showed a lowering of the pressure of the cerebro-spinal fluid to below zero. This animal was given an intravenous injection of 10 cc. of the saturated solution. During the first part of the injection the pressure of the cerebro-spinal fluid rose 40 mm. but before the completion of the injection a rapid fall had begun. This break in the initial rise before the completion of injection is recorded in most of the curves with this salt. The marked fall continued abruptly in this case to 12 mm. below zero, a point reached in sixteen minutes after the end of the injection. This pressure remained at zero or below for a period of forty minutes and then slowly started upwards. This curve is reproduced in figure 8.

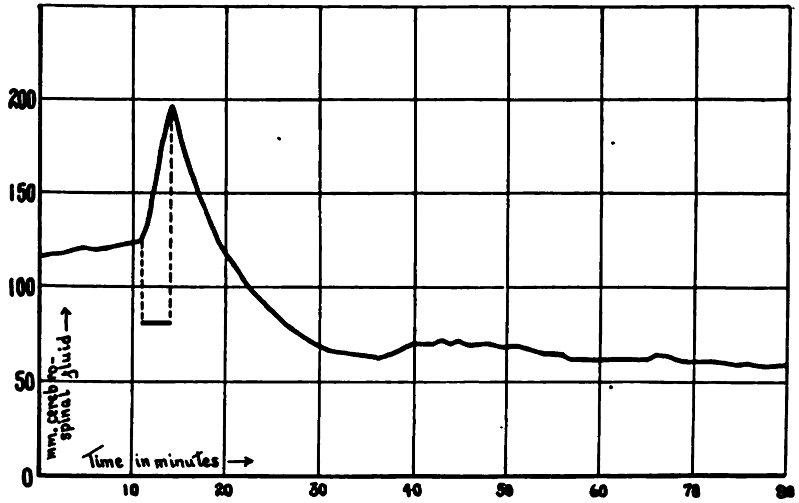

Fig. 7. Cat no. 1395. Pressure cerebro-spinal fluid with intravenous injection of 10 cc. 30 per cent sodium chloride.

The more common response in the pressure of the cerebro-spinal fluid to intravenous injection of sodium bicarbonate is merely a lowering of 40 to 70 mm. The curves show typical peaks with breaks before

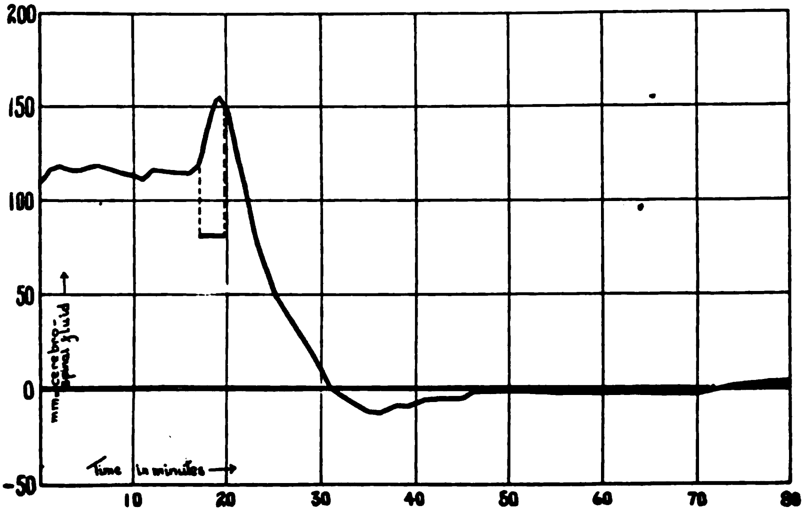

Fig. 8. Cat no. 1364. Pressure cerebro-spinal fluid with intravenous injection of 10 cc. saturated solution of sodium bicarbonate.

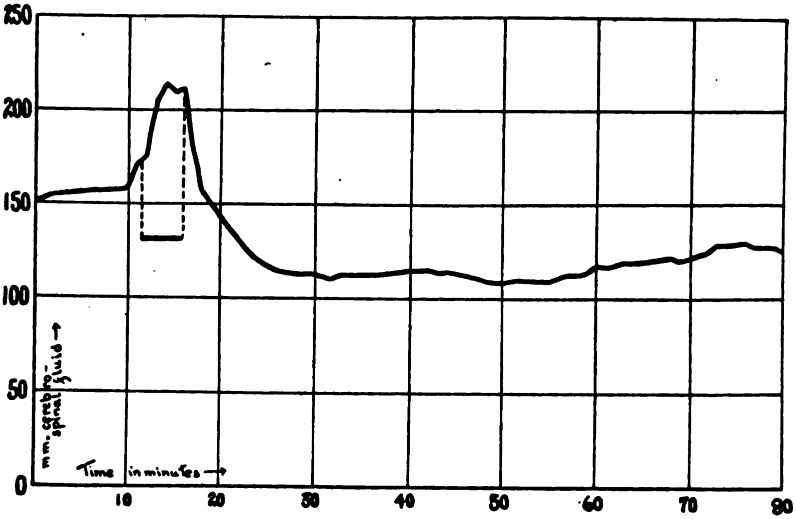

Fig. 9. Cat no. 1365. Pressure cerebro-spinal fluid with intravenous injection of 18 cc. saturated solution of sodium bicarbonate.

the completion of the injections. One of these typical curves with a lowering of the pressure of the cerebro-spinal fluid of approximately 40 mm. is given in figure 9. The injection in this case was 18 cc. of the saturated solution of the bicarbonate; the difference in reaction to a smaller dose in another animal is shown by comparison with figure 8. At the end of the period of observation some recovery toward the normal pressure is shown in figure 9.

Sodium sulphate. Four experiments with the intravenous injection of a 30 per cent solution of sodium sulphate were carried out. In all of these observations a reduction of pressure of the cerebro-spinal fluid was recorded, the fall being usually 50 to 80 mm. A typical initial rise in the pressure during the period of injection with formation of a peak is shown by all the curves, one of which is reproduced in figure 10. In this case an injection of 10 cc. of the 30 per cent solution of sodium sulphate was given; this gave an abrupt initial rise of 80 mm. which was promptly followed by a rapid fall to a point about 90 mm. below the pressure at the beginning of the experiment. Subsequently a low pressure (about 70 mm.) was maintained for thirty minutes with a gradual rise but incomplete recovery in pressure. Such a curve is typical of the series; none have shown pressures below zero.

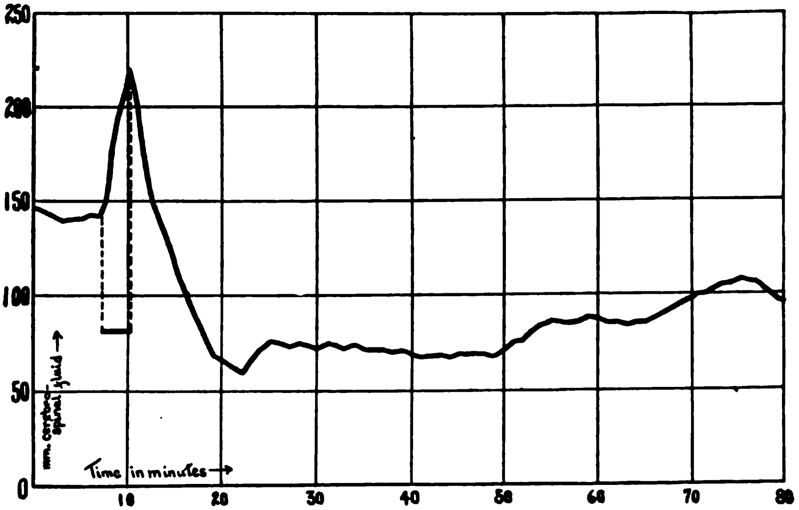

Fig. 10. Cat no. 1356. Pressure cerebro-spinal fluid with intravenous injection of 10 cc. 30 per cent sodium sulphate.

Glucose. The observations in the foregoing paragraphs of this section have all been concerned with the lowering of the pressure of the cerebro-spinal fluid by the intravenous injection of concentrated solutions of electrolytes (common sodium salts). In an attempt to determine whether all crystalloids possess this power of bringing about a lowering of the pressure of the cerebro-spinal fluid, four experiments were undertaken in which intravenous injections of a concentrated solution of glucose were given. Large amounts of a 30 per cent solution were given and typical responses were obtained. The curves all show a marked abrupt initial rise followed quickly by a fall to a level lower than that prevailing at the beginning of the experiment. This lowering was not as marked as with the electrolytes but in two of the experiments the resultant level was 60 mm. below the initial pressure.

Toxicity and dosage of the sodium salts. It was early noticed that the intravenous injection of the concentrated solutions of sodium chloride was followed by severe respiratory and cardiac disturbances. It was often necessary to alter the anesthetic to avoid losing the animal. The ether was in these cases reduced and artificial respiration used, the intratracheal method of anesthesia making possible a nearly ideal artificial respiration. This toxicity of the sodium chloride was apparently not dependent upon the absolute amount injected but was frequently observed in the earliest intervals of the injection when only 1 to 2 cc. had been introduced. As soon as this initial toxicity was passed, further injection up to certain limits could be made with comparative safety in the same animal. Such early disturbances seem best explained on the basis of an alteration in the balance of the mineral salts in the blood.

In addition to the initial toxicity of the sodium chloride, another factor of importance in these experiments was variation in the tolerance of the individual animal. Some animals, showing no apparent disturbance on receiving relatively large intravenous injections, have given marked reactions in the pressure of the cerebro-spinal fluid following such injections. Other animals under similar conditions have exhibited but little reaction as indicated by a lowering of the pressure of the cerebro-spinal fluid. In still other animals where the toxic effects of the salt were marked, the lowering of the pressure of the cerebro-spinal fluid was definite and considerable. Thus an individual tolerance and reaction for each particular animal seems indicated, a tolerance and reaction which we have not been able to predict in our experiments.

Quite similar to these experiences with intravenous injections of sodium chloride were our findings with the bicarbonate and sulphate. It has already been pointed out that in one case the injection of 10 cc. of the saturated bicarbonate gave a far greater fall in the pressure of the cerebro-spinal fluid than an injection of 18 cc. of the same salt in another animal (cf. figs. 8 and 9). Such individual variations are common also with the sulphate, although the final toxicity of the salt used in our experiments seems much greater. Few animals have shown any disturbance during the injection of the sulphate solution but several have died, apparently from the toxic effect of the salt, before the completion of the observation.

Following the intravenous injection of these concentrated salts, then, the character of the resultant curve, as indicating the extent of the reaction producing the lowering of the pressure of the cerebro-spinal fluid, must be referred partially at least to the individual tolerance and reaction of the animal used in the experiment. The definite and marked lowering of the pressure by the intravenous injection of concentrated solutions of electrolytes is shown in all the curves, but the degree of reaction seems dependent, to some extent at least, on the individual animal. All of these animals, if allowed to recover from the ether, seem slow and quiet for a few hours; within twelve hours they become normal in every way.

DISCUSSION

In a foregoing part of this paper the results of the reading of the pressure of the cerebro-spinal fluid in sixty-five cats have been set forth. The average of the initial readings in these cases was 119 mm. but it was pointed out that if readings were taken a few minutes later, a higher value (129 mm.) was obtained. This higher value, as has already been indicated, is probably to be accounted for by replacement of fluid which follows the dislocation and loss due to the mechanical arrangement of the experiments. It seems reasonable to assume then, that, after sufficient time has elapsed to allow replacement of fluid which occurs after the puncture and connection of the manometer, we are dealing with a pressure of the cerebro-spinal fluid which is truer normal than would be obtained by readings taken immediately after puncture.

There are available in the literature records of observations on the pressure of the cerebro-spinal fluid which have been made on dogs under various anesthetics and by various methods. So far as is known to us, no observations of this sort have been made on cats. In general, our readings on cats agree fairly well with those recorded for dogs. They are somewhat lower than those given by Key and Retzius (5) (162 to 216 mm. of water in inspiration; 216 to 270 mm. of water in expiration—dogs under ether); higher than Bergmann's first observations (6) (80 mm. of water, narcotized dogs) but agreeing fairly well with his later findings (7) (120 to 160 mm. salt solution, narcotized dogs). They coincide fairly accurately with the records of the work of Falkenheim and Naunyn (8) (100 to 150 mm. of water, dogs curarized), and while the conditions in Leyden's experiments (9) are not comparable with those prevailing in ours, his results (80 to 150 mm. of water, 100 to 120 mm. of water, dogs under morphia) seem well in accord with ours. It is felt that the method of intratracheal anesthesia and the direct readings of the pressures in the manometer as practiced in our experiments give a better index of the normal than was obtained by Dixon and Halliburton using a graphic method and morphine-urethane anesthesia. Dixon and Halliburton (10) give a rough average of 40 to 70 mm. of salt solution as the normal pressure of the cerebro-spinal fluid in the dog, but the extreme variations which they record have not been noted in our observations on control animals. Thus they record as typical variations of the normal pressure of the cerebro-spinal fluid in the dog, the following values read at five-minute intervals: 95 – 25 – 30 – 35 – 55 – 25 – 80 – 65 – 65 – 75 – 70 – 60 – 55 –50 – 80 – 90 mm. of 10 per cent sodium citrate solution. Compared to the curve reproduced in figure 2, in which the respiratory and cardiac excursions were well marked throughout, these variations recorded by Dixon and Halliburton seem extreme.

The logical physiological explanation of the alterations in the pressure of the cerebro-spinal fluid following the injection of hypertonic and hypotonic solutions, seems naturally to be concerned with the experimental change in the osmotic value of the blood. Thus an increase in the concentration of the fluids injected resulted in a fall in the pressure of the cerebro-spinal fluid while the injection of a hypotonic solution gave an enduring rise. Data regarding the relative or absolute osmotic values of the blood have not been obtained. It would appear that the alteration in the salt content of the blood produced experimentally can only be compensated by fluid readjustments within the tissues, for renal function is more or less impaired by the anesthesia. The individual reaction of the animal noted above may therefore be referred to variation in the fluid interchange between blood and tissue, so that alterations in the pressure of the cerebro-spinal fluid following intravenous injections of hypertonic and hypotonic solutions seem dependent on the osmotic readjustments within the tissues.

A number of observations have been made on the pressure of the cerebro-spinal fluid with simultaneous record of the arterial blood pressure, heart-rate and venous pressures. Further experiments of this kind are under way and will form the basis of a future report.

SUMMARY

1. The pressure of the cerebro-spinal fluid in the etherized cats has been found to average 119 mm. of this fluid, if read immediately after the connection of the manometer with a needle in the subarachnoid space; but this pressure, if read some minutes later, is somewhat higher, giving an average of 129 mm., this rise being due, partially at least, to the replacement of cerebro-spinal fluid displaced in the manometer or lost during its connection.
2. Intravenous injections of Ringer's solution cause no lasting change in the pressure of the cerebro-spinal fluid.
3. Intravenous injections of hypotonic solutions (distilled water) are followed by a marked and sustained rise in the pressure of the cerebro-spinal fluid.
4. Intravenous injections of hypertonic solutions (concentrated sodium chloride, sodium bicarbonate, sodium sulphate and glucose) cause initial rise in the pressure of the cerebro-spinal fluid followed immediately by marked fall in this pressure, often to below zero.

BIBLIOGRAPHY

(1) CAPPELLETTI: Arch. Ital. d. Biol., 1900, xxxv, part 2, 463.
(2) PETTIT AND GIRARD: Sur la fonction secretoire et la morphologie des Plexus Choroides des ventricules lateraux du systeme nerveux, Paris, 1902.
(3) DANDY AND BLACKFAN: Amer. Journ. Diseases Children, 1914, viii, 406.
(4) DIXON AND HALLIBURTON: Journ. Physiol., 1913, xlvii, 215.
(5) KEY AND RETZIUS: Anatomie des Nervensystems und des Bindesgewebe, Stockholm, 1876.
(6) BERGMANN: Deutsch. Chirurgie, 1880, xxx, 266.
(7) BERGMANN: Arch. f. klin. Chirurgie, 1885, xxxii, 705.
(8) FALKENHEIM AND NAUNYN: Arch. exper. Path. u. Pharm., 1887, xxii, 261.
(9) LEYDEN: Arch. f. path. Anat. u. Physiol., 1866, xxxvii, 519.
(10) DIXON AND HALLIBURTON: Journ. Physiol., 1914, xlviii, 128.

EXPERIMENTAL ALTERATION OF BRAIN BULK

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In the early stages of an investigation of the factors underlying the swelling (edema) of the brain in acute infections or injuries, attention was directed to the possible relationship between the volume of the brain and the alteration in the pressure of the cerebro-spinal fluid, following intravenous injections of solutions of various concentrations (1). For in the study of cerebral edema, but little progress has in the past been made on account of the difficulty of experimental approach. This condition remains today one of the great problems in pathology of the central nervous system.

The marked changes in the pressure of the cerebro-spinal fluid, reported in the foregoing paper, were quickly found to have a definite relation to the resultant volume of the brain. Thus, following intravenous injections of strongly hypertonic solutions which markedly lowered the pressure of the cerebro-spinal fluid, definite shrinking of the brain occurred. And conversely the brain bulk was appreciably increased by the intravenous injection of hypotonic solutions, which raised the pressure of the cerebro-spinal fluid. Such changes in the size of the brain are rapidly and uniformly brought about, giving definite information as to one phase of the physiological regulation of the volume of this organ.

METHODS

Cats were used entirely in this work. Intravenous injections of the various solutions were given with a syringe or with a burette connected directly with a fore-leg vein. For the hypertonic solutions, 30 per cent sodium chloride or saturated sodium bicarbonate in distilled water were given, as previous work had demonstrated their efficacy in lowering the pressure of the cerebro-spinal fluid. Ringer's solution (NaCl, 0.9 per cent; KCl, 0.042 per cent; $CaCl_2$, 0.025 per cent) was injected intravenously to give data regarding the possible alteration in the circulation and in the volume of the brain brought about by the introduction of an increased volume of fluid, while distilled water was used as the hypotonic solution. Cats which had been given the customary intravenous injections of these solutions and then allowed to recover from the anesthetic, were usually a little slow for six hours but became normal and active within twelve hours. For the most part the observations were carried out on cats with unopened skulls, but in two series, subtemporal trephine openings were made, not only to relieve the intracranial tension but also to permit direct observation of the brain.

All animals used in these observations were anesthetized with ether, usually by intratracheal insufflation but in the earlier experiments by cone. The body temperature of the animals was maintained throughout. After the lapse of time necessary for the maximum action of the solution intravenously introduced, the animals were killed by ether. In the routine experiment, 10 per cent formalin was injected, immediately after death, through the aorta at a pressure of not more than 800 mm. of water. When the cranial vessels were well filled, the central nervous system was removed (the skull and vertebral canal being partially opened) and the whole immersed in 10 per cent formalin. In spite of all the care it was possible to exercise, it was soon very evident that by this method of fixation, the form and size relations of the central nervous system prevailing prior to the death of the animal were not being accurately preserved. Brains markedly shrunken during life or at death of the animal approached almost normal proportions after such fixation, and brains markedly herniated at death often subsided perceptibly during preservation. The brains of other animals were fixed by direct immersion in formalin; the results of this method were similar in regard to alteration in volume. The addition of a suitable amount of sodium chloride to the formalin solution did not prevent these changes in brain bulk during preservation and was as unsatisfactory as other solutions.

Although the method of fixation used is inadequate, still it enables one to make general comparisons of the brains after various intravenous injections, even though it does not preserve the volume relations accurately. It is hoped there will be found a means of fixation which will preserve more exactly the form and size relations and at the same time make possible a study of the histology and cytology of these brains with reasonable confidence.

EXPERIMENTS WITH UNOPENED SKULL

The majority of these experiments were carried out on animals without opening of the skull. Some of this series, however, were used for determinations of the pressure of the cerebro-spinal fluid; in these the subarachnoid space was entered by a needle through the occipito-atlantoid ligament for connection with a manometer. In this limited manner the pressure relations within the cranium may be considered to have been altered; other animals of this series were carried through with intact cranial cavities.

Normal. Under this heading it is purposed to discuss the normal bulk of brains as found in cats killed without experimentation, and in those given Ringer's solution in such amounts as to control the volume of the other intravenous injections. The intravenous injections of Ringer's solution in these quantities did not alter the volume or appearance of the brain, so that, as far as our observations go, the brains of these animals are to be classed as normal.

When removed from the skull after routine fixation with formalin, the normal brain surrounded by unopened dura presents in the cat typical appearances. The dura over the convexities is only fairly well filled out and is under no appreciable tension, falling slightly between the adjacent gyri. On looking through the dura, a definite rounding of the convolutions and the fairly well-defined edges of the sulci are apparent. On transverse section of the normal brain (fig. 3), differentiation between gray and white matter is obvious. At the periphery of the section, the dura is seen to fall slightly between adjacent gyri. The surfaces of the gyri present smoothly-rounded curves, dipping into well-defined sulci of appreciable width. The median longitudinal fissure is clear cut and the adjacent gyri definitely separated. A line formed by the arachnoid membrane can be made out, bridging the larger sulci. Quite similar appearances are presented by the brains of animals receiving intravenous injections of Ringer's solution (fig. 4).

Examination of a large series of cats' brains fixed under similar conditions has shown that considerable individual variation exists. In brains of old animals, the gyri appear more rounded and the sulci deeper than in the younger cats. The dura in such older animals seems looser and denser, suggesting the various phenomena of old age exhibited by the brain in man. In very young cats and kittens, there seems to be a tendency toward swelling following formalin fixation. These individual variations, according to age, must be constantly borne in mind while interpreting the results of the experimental modification of the volume of the brain.

Hypotonic solutions. The intravenous injection of water, which has been found to produce a definite increase in the pressure of the cerebro-spinal fluid, causes also a frank swelling of the substance of the brain. Amounts varying from 20 cc. to 100 cc. were injected intravenously; the degree of the reaction was apparently not dependent upon the absolute quantity of water injected, for the maximum effect observed occurred in a cat receiving only 20 cc. of water intravenously. Figure 1 gives the gross appearance of the formalinized brain of a cat which had been subjected to an intravenous injection of 35 cc. of distilled water, and sacrificed thirty-five minutes after completion of injection. The dura over the cerebral hemispheres is markedly tense as in all others similarly treated. The convolutions appear flattened when viewed through the dura and the sulci are traced with greater difficulty than in the normal. On passing the finger over the dura covering the upper surface of the brain, one receives an impression of marked tenseness of dura and brain, and recognizes the gyri and intervening sulci with difficulty.

On section, such brains (figs. 5 and 7) exhibit the same tenseness of dura previously noted. The normal differentiation between the gray and white matter has been diminished (figs. 3, 5 or 7). The convolutions appear definitely flattened, adjacent gyri being pushed together so as to make the identification of the intervening sulci difficult. This is particularly true of the smaller sulci. The surfaces of the gyri are no longer gently convex but acute angles in the curve are found where the surface dips into the sulci. The superior longitudinal fissure is narrow and the bounding gyri press tightly against the falx. The cut surface of the brain appears definitely turgid and gives the impression of having been subjected to increased tension.

An increase in the volume of the brain following the intravenous injection of water is therefore definite, marked and readily apparent.

Hypertonic solutions. The intravenous injection of strongly hypertonic solutions, which has been found to cause a profound lowering of the pressure of the cerebro-spinal fluid, has been observed to produce also a decrease in the bulk of the brain. This alteration in the volume of the brain has been brought about by intravenous injections of from 8 cc. to 20 cc. of 30 per cent sodium chloride or saturated sodium bicarbonate. As reported elsewhere under the subject of alterations in the cerebro-spinal fluid pressure, a marked individual variation in reaction to hypertonic solutions has been found to exist. The dose bringing about the maximal cerebral shrinkage varied, but in general the larger doses, approaching the limit of the animal's tolerance, seemed to cause the most marked effect. Some of the cats were given single injections of as much of the hypertonic solution as they seemed able to stand at one administration. Other animals were given a series of 5 cc. doses of 30 per cent sodium chloride at half-hour intervals, until a total of 20 cc. was injected. The animals, except when given the divided doses at half-hour intervals, were kept under ether anesthesia until sacrificed.

The time necessary for the maximum action of the fluid injected could not be determined accurately for the cats with unopened skulls. Assuming however, that the maximum lowering of the pressure of the cerebro-spinal fluid after such an injection coincides with the maximal diminution of brain-volume, it is probable that an interval of fifteen to twenty minutes suffices for the maximum change. Within certain limits, these observations substantiate this assumption, namely that the amount of fall in pressure of the cerebro-spinal fluid is an index of the extent to which the volume of the brain has been reduced.

The brain of a cat after the intravenous injection of a strongly hypertonic solution shows, on routine formalin fixation, a marked decrease in volume. As seen through the dura (fig. 2), which is very loosely applied, the brain seems comparatively small, occupying only a part of the intradural space. The gyri appear markedly rounded and the sulci wide and deep, so that individual convolutions appear throughout their extent. In the medulla oblongata and spinal cord there is also evidence of marked decrease in size. The dura here is very much more loosely applied than in the normal and the markings of the medulla oblongata and spinal cord seem sharp and accentuated. The general impression received from such an uncut brain is that it is quite small in comparison to the dural sac.

When cut transversely, the brain from a cat subjected to this experimental procedure presents an appearance quite different from the normal. The gray and white matter are far more sharply contrasted. Furthermore, the gray matter, particularly that of the thalamus and corpus striatum, appears dark with a brownish tinge, clearly outlining the nuclei from the adjacent white fibers. This phenomenon has been noted quite uniformly in this series. On such a section, the dura is very loosely applied (fig. 6), touching on the dorsal surface only the highest points of the gyri. Each individual gyrus stands out cleanly separated from adjacent gyri by widely opened sulci. The curve presented by the upper surface of each gyrus is of smaller radius than the normal, and may be followed deeply into each sulcus. The superior longitudinal fissure gapes widely and the falx seems to hang loosely within this space.

Similar shrunken brains have been obtained by the intravenous injection of saturated solutions of sodium bicarbonate. A section of such a brain is shown in figure 8, which presents in general the characteristic features noted above. The decrease in volume is, however, not so striking as in the brain reproduced in figure 6.

It was thought possible that the action of these hypertonic solutions might be enhanced by depriving such animals of all fluid for a sufficient length of time before experimentation to insure the exhaustion of a considerable quantity of the water available in the body. Two series of animals were thus prepared and injected with 30 per cent sodium chloride in 5 cc. doses at one-half hour intervals. While the cats receiving four 5 cc. doses showed the effects of the injection in a very marked way (gross clonus of whole body, mild mania, etc.), the brains failed to show any more, if as much shrinkage as is shown by the brains of animals not denied water. These observations indicate that deprivation of water for twenty-four hours in the cat is not sufficient to alter the fluid-volume of the body tissues available for reaction with hypertonic solutions.

In the unopened skull, then, a definite decrease in the bulk of the brain may be brought about by intravenous injection of strongly hypertonic solutions.

The supply of a foreign solution to subarachnoid space. At the end of a number of the experiments in which an intravenous injection of hypertonic sodium chloride was given, a mixture of sodium ferrocyanide and iron-ammonium citrate was allowed to flow into the subarachnoid space. This was done at a time when the pressure of the cerebrospinal fluid was about zero or falling rapidly. Two or three cubic centimeters were usually so introduced. At the end of the experiment the animal was injected through the aorta with 10 per cent formalin, to which 5 per cent hydrochloric acid had been added, and when the vessels were well filled the central nervous system was quickly removed and immersed in the acid formaldehyde. By this procedure, Prussian blue was precipitated at the points to which the solutions of sodium ferrocyanide and iron-ammonium citrate had penetrated prior to fixation. The Prussian blue in almost every case was found to have passed from the subarachnoid space along the perivasculars into the substance of the nervous system, reaching the interfibrous spaces in the white matter and the pericellular spaces in the gray. These observations may be interpreted as indicating that the hypertonic solution of sodium chloride, injected intravenously, had caused the dislocation of a considerable quantity of the cerebro-spinal fluid into the nervous system.

EXPERIMENTS WITH THE OPENED SKULL

The experiments dealing with the alteration of brain bulk by intravenous injection of solutions of varying concentrations were, in the earlier part of this investigation, carried out with the unopened cranial cavity, in which a fairly constant fluid volume was necessarily maintained. Even with such limitation to change in the volume of the brain, these solutions of various tonicities caused marked modification in its size. It was therefore thought desirable to carry out similar observations but with opening of the skull to permit expansion or contraction of the brain—changes impossible under the physical conditions imposed by the closed cranium. The rate of reaction to the intravenous injection and the appearance of the brain throughout the experiment could also, under these conditions, be determined by direct observation.

The opening of the skull was accomplished in the subtemporal region. In the etherized animal, through a midline incision, the temporal muscle on one side was freed from its origin, and a trephine opening of 2 cm. made in the skull beneath the muscle. The upper border of this opening came within 3 mm. of the mid-sagittal line of the skull. In another series bilateral subtemporal decompression openings were made. In every case the dura was freely opened by cruciate incisions. Injury to the underlying arachnoid and brain was carefully avoided.

Normal. Control experiments with single and bilateral decompressions were carried out. Following the opening of the skull, the animal was kept under ether until sacrificed at the expiration of about the same length of time as was consumed in the experiments where intravenous injections were given. In these control animals under such conditions the brain lay slightly convex beneath the trephine opening, pulsating freely, and did not change perceptibly in any way during the period of observation. The dural flaps were allowed to lie loosely on the exposed surface of the brain and throughout the experiment the edges of these flaps were separated from 1 to 2 mm. in the center of the trephine opening. At the end of these observations the animals were sacrificed with ether and the brains immediately preserved in 10 per cent formalin. On section, these brains appear quite normal with evidence of slight dislocation of brain substance toward the site of the trephine openings. Figure 13 shows a section of such a brain which was relieved by a single opening in the skull and dura; figure 16 represents the condition prevailing after a bilateral subtemporal decompression. It may be concluded, then, that under the conditions of experimentation the volume of the brain has been but little changed by the anesthesia and operative procedures employed.

Further control of the observations of brain bulk, following intravenous injection of solutions of various concentrations, is afforded by several experiments in which Ringer's solution was injected intravenously after single or bilateral openings had been made in the skull and dura. The injection of Ringer's solution was from a burette, and the rate of fluid-introduction was regulated to coincide with that used in the injection of similar amounts of hypotonic solutions. During and after the injection of Ringer's solution, in amounts up to 100 cc., the brain lay slightly convex in the trephine openings, pulsating freely, and showed no evidence that it had been affected in volume by the intravenous injection. The appearance of the cortex viewed through the trephine opening was exactly that of the brain of an animal receiving no intravenous injection, but subjected to the other operative procedures. In figure 10 is shown the result (after fixation in formalin) of an experiment in which a single opening was made in the skull and 100 cc. of Ringer's solution injected intravenously. As pointed out previously, we have been unable with our present methods to preserve, by fixation in formaldehyde, the form and size relations prevailing in the brain at the end of experimentation. This figure shows a slightly more marked bulging of the brain in the trephine opening than was present at the end of the experiment. In spite of this slight swelling due to fixation it presents a fairly normal appearance, particularly when compared with more swollen or shrunken brains as shown in figures 9 and 11. In this and in all of our observations in which Ringer's solution was injected, the anesthesia, the operative procedures, the time consumed by the intravenous injection and the interval of time from the end of the injection to the sacrifice of the animal, were similar to those in the experiments in which solutions of various concentration were injected.

It may be concluded, then, that in etherized animals with the skull opened, the intravenous injection of Ringer's solution in amounts up to 100 cc. causes no appreciable change in the volume of the brain. The protocol of a typical experiment in which there was a bilateral opening of the skull and the intravenous injection of 100 cc. of Ringer's solution, is given below:

No. 1537. Adult male cat. Weight 2500 grams. Control intravenous Ringer.

9.46 a.m. Ether with intratracheal tube.
10.15 a.m. Double subtemporal decompression. Dura opened. Brain lies with normal convexity, pulsation and circulation good.
10.20 a.m. Cannula in vein of fore-leg connected with burette containing Ringer's solution.
10.25 a.m. Injection begun. Brain as before.
10.33 a.m. 25 cc. in. Brain as before.
10.38 a.m. 50 cc. in. Brain slightly more convex.
10.45 a.m. 90 cc. in. Brain as before.
10.50 a.m. 100 cc. in. Brain normally convex, shows no bulging. Pulsation free, circulation good. Injection stopped.
11.10 a.m. Brain lies normal as before. Pulsation free.
11.26 a.m. Brain lies normal as before. Ether to death. Immediately injected with 10 per cent formaldehyde until vessels well filled, then head cut off and immersed in same solution. Original relations fairly well preserved after fixation.

With the control afforded by these experiments in which the skull was opened and in which there was no intravenous injection, or else the introduction of Ringer's solution, an interpretation of the results of the intravenous injection of hypotonic and hypertonic solutions may be safely attempted.

Water. A number of observations on cats with single and bilateral openings of the skull have been made, during and following the intravenous injection of sterile distilled water which, as noted in a previous section of this paper, has been shown to bring about an increase in the volume of the brain in the unopened skull. The conditions prevailing in these experiments were similar to those maintained during the injection of Ringer's solution. In all these animals the brain, which lay normally convex in the trephine openings, began to protrude very soon after the intravenous injection of distilled water was started. This bulging increased throughout the period of injection and reached its maximum usually in from ten to twenty minutes after the completion of the introduction of water. Tense herniae of the brain through the trephine openings were thus produced. The tension was in all cases so great that cerebral pulsation ceased before the swelling reached its maximum. The pressure of the brain on the dura at the edges of the trephine openings was usually marked enough to stop the circulation in the dural flaps. These triangular flaps were stretched, pulled and rolled back into the interval between the hernia and the cut edge of the trephine opening. Figure 9 shows the result of such an experiment and presents fairly well the appearance of the cerebral hernia before the sacrifice of the animal, although fixation in formaldehyde at death of the animal caused some subsidence of the hernia. When observed just before the death of the cat, usually thirty minutes after the completion of the injection of water, the brain protruded, in most of our experiments, at least 4 mm. beyond the outer table of the skull; in one animal which gave a very marked reaction, the height of the hernia at the end of the observation was 8 mm.

The protocol of an experiment in which there was a bilateral opening of the skull and the intravenous injection of 100 cc. of sterile distilled water, is given below:

No. 1536. Adult female cat. Weight 2,430 grams. Intravenous water

3.50 p.m. Ether with intratracheal tube.
4.00 p.m. Double subtemporal decompression.
4.15 p.m. Dura opened. Brain lies with normal convexity, pulsating freely. No trauma to brain or membranes.
4.18 p.m. Cannula in vein of fore-leg connected with burette containing sterile distilled water.
4.19 p.m. Injection begun. Brain bulges immediately after beginning of injection.
4.25 p.m. Brain bulges more.
4.31 p.m. 50 cc. in. Brain bulges markedly with pulsation; circulation good.
4.35 p.m. 75 cc. in. Brain bulges markedly. Pulsation slight, circulation good.
4.45 p.m. 100 cc. in. Injection stopped. Brain in tense hernia. Pulsation slight on left side. No pulsation on right side.
5.00 p.m. Brain markedly herniated. No pulsation on either side.
5.15 p.m. Brain in tense hernia on both sides—stops circulation in dural flaps.
5.16 p.m. Ether to death. Immediately injected through the heart with 10 per cent formalin and when vessels filled, head cut off and immersed in same solution. After immersion hernia remains about as before. Convolutions slightly more rounded and whole hernia slightly flatter.

On section, these brains present the appearance seen in figure 12 (after single decompression) and in figure 15 (after bilateral opening of the skull). A considerable dislocation of brain substance is apparent with some flattening of the gyri and narrowing of the sulci; but the general impression gained from an examination of these sections is different from that resulting from observation of sections, as shown in figures 5 and 7, of brains obtained from animals subjected to the intravenous injection of water, but with unopened skulls. In the sections of the brains which were allowed to herniate, the impression of extreme tenseness and turgidity is not outstanding as is the case with the brains which were restrained by unopened dura and skull. The difference is undoubtedly to be explained by the different mechanical limitations to expansion present in the two types of experiment.

It is apparent from the foregoing that, under experimental conditions similar to those prevailing in the control observations on brains with opened skull (with or without the intravenous injection of Ringer's solution), the intravenous introduction of water causes a marked herniation of cerebral substance resulting from the increase in the volume of the brain. It is also worthy of note that the gross appearance of sections of such brains is different from that previously observed in sections of brains of animals receiving water intravenously but with unopened skulls.

Salt. Intravenous injections of a hypertonic solution (30 per cent sodium chloride), which produced a marked reduction in volume of the brain in animals with unopened skulls, have been given to a number of cats in which openings in the skull and dura were made on one or both sides. The conditions prevailing in these experiments were similar to those already described for the control observations. Following the intravenous injection of 30 per cent sodium chloride, the normal convexity of the brain in the trephine opening disappears soon after the injection is begun, so that the brain is seen to lie flat. As the intravenous injection of the salt is continued, the brain falls away from the skull until the surface presented becomes concave. The maximum shrinkage has been observed usually in from fifteen to thirty minutes after the completion of the injection, when the brain lies flaccid, 3 to 4 mm. below the inner table of the skull, with only very slight visible pulsation. In figure 11 is shown the result of an experiment in which a single opening was made in the skull, and 16½ cc. of a solution of 30 per cent sodium chloride injected intravenously. The photograph reproduced in this figure was taken after the fixation of the head in 10 per cent formalin and does not show the marked shrinkage which was so striking at the end of the experiment. As has been emphasized, the methods of fixation employed do not preserve, with the accuracy desired, the relations existing during life; in spite of this difficulty it is readily apparent, from figure 11, that here the skull is only partially filled by this markedly shrunken brain.

The individual reaction and tolerance of cats to intravenous injections of a hypertonic solution of sodium chloride and the quantities most effective in producing a decrease in the volume of the brain, have been discussed in a preceding section of this paper. In these observations with the opened skull, doses approaching the limit of the animal's tolerance (16 to 20 cc.) have been administered; the same differences in individual reaction and tolerance, noted before, have been observed.

There is given below the protocol of a typical experiment in this series, in which there was a bilateral opening of the skull and dura and an intravenous injection of 20 cc. of a hypertonic solution of sodium chloride:

No. 1535. Adult female cat. Weight 2250 grams. Intravenous NaCl.

9.35 a.m. Ether with intratracheal tube.

10.10 a.m. Double subtemporal decompression.

10.15 a.m. Dura opened. Brain lies with normal convexity, pulsating freely. No injury to brain or membranes.

10.20 a.m. Cannula put in vein of fore-leg and connected with burette containing 30 per cent NaCl (Squibb).

10.23 a.m. Injection begun. Convexity of brain normal.

10.30 a.m. Brain as before. No hernia.

10.35 a.m. 11 cc. in. Both sides of brain receding—lie flat.

10.40 a.m. 14 cc. in. Brain fallen more—lies concave.

10.45 a.m. 16 cc. in. Brain fallen more.

10.50 a.m. 19 cc. in. Brain fallen still more.

10.54 a.m. 20 cc. in. Injection stopped. Brain markedly fallen. Circulation good, pulsation slight.

11.05 a.m. Brain markedly shrunk. Pulsation slight. Animal in good shape.

11.25 a.m. Brain far receded, pulsation slight. Brain lies 3 to 4 mm. away from the inner table of the skull.

11.26 a.m. Ether to death. Immediately injected through the heart with 10 per cent formaldehyde plus 1.5 per cent NaCl with cat lying on belly. Head cut off when vessels well filled and immersed in same solution. Within a few minutes after immersion brain rose in skull almost level with trephine opening.

After fixation in formalin and section, these brains, taken from animals with opened skulls and with intravenous injection of 30 per cent sodium chloride, are characterized by the condition shown in figure 14 (single skull opening) and figure 17 (bilateral subtemporal decompression). That there has been a decided decrease in the volume of the brain in both these cases is quite evident. Figures 12, 13 and 14 show sections of a series of brains taken from animals in which there was a single subtemporal decompression. In one animal (fig. 12) water was injected intravenously; in another (fig. 14) 30 per cent sodium chloride, while in the third, (fig. 13) no injection was given. Figures 15, 16 and 17 show sections of brains from another series of animals in which bilateral openings were made in the skull and dura. Intravenous water was given in one animal (fig. 15), intravenous 30 per cent sodium chloride in another (fig. 17), but in the control (fig. 16) nothing was injected. A glance at these figures is quite sufficient to show that in the animal receiving the intravenous injection of a solution of hypertonic sodium chloride in both series, the brain has been markedly decreased in volume. A close examination of figures 14 and 17, and a comparison of these figures with figures 6 and 8, which resulted from experiments in which hypertonic solutions were injected intravenously but with the skull unopened, is extremely interesting in that, while there is evident shrinkage in both types of experiment, the way in which the brain was affected is different in the two. From figures 14 and 17 (opened skull) one gets an impression of marked compactness of the brain as a whole; this phenomenon is not so apparent in figures 6 and 8 (unopened skull). The well rounded gyri and the clearly apparent sulci, previously described as characteristic (following intravenous injections of hypertonic solutions) of brains arising from experiments in which the skull was unopened, are not to be found in brains after similar experimental procedures but with the skull opened. It is evident, then, that with the operation of the same factor which tends to produce a decrease in the volume of the brain, the form of the end result in the two cases is altered by the mechanical conditions imposed by the opened or unopened skull. It is thus apparent that when the brain is allowed by an opened skull to shrink and contract freely, the appearance of a greater decrease in total volume is obtained than in experiments where the force producing the reduction in volume must, as it were, pull against a partial vacuum furnished by the intact skull.

These observations make it clear that in brains unrestrained by the physical limitation of the closed cranium there is a marked decrease in volume after intravenous injection of hypertonic solutions of sodium chloride; but the resulting picture is different from that described for brains shrunken, after similar injections, within an intact skull.

In the course of the above experiments the failure of the brain of a very old cat to show marked swelling after the intravenous injection of water led to several observations on decidedly old animals. Two very old cats, following double temporal decompression, were given intravenous injections of 100 cc. of water. The brain of neither cat herniated markedly from increase in bulk. Another old cat, after double temporal decompression, was given an intravenous injection of 20 cc. of 30 per cent sodium chloride. The brain shrank away from the skull to a considerable extent, although not so far as is usually the case in younger individuals. It has been frequently noted that when exposed to view by operative procedure, the brains of these old cats look different from those of younger individuals. While they pulsate freely in the decompression openings, they lie flat and do not show so much convexity as is characteristic of the brains of younger individuals. The sulci of these brains of old cats seem wider and the gyri more rounded than those characteristic of younger cats. That the brains of these old animals react less readily to intravenous injections by changes in bulk than the brains of younger individuals seems certain from these observations; that increase in volume should be more difficult than decrease seems reasonable, in view of certain mechanical and other conditions existing within the cranium in old individuals.

Two very young cats weighing 1,300 and 1,500 grams, were selected as typical young adults, in which changes in brain bulk should be outspoken. Following double subtemporal decompression, one received an intravenous injection of 100 cc. of water, the other 16 cc. of 30 per cent NaCl. Both showed very well marked reactions, developing relatively as great swelling or shrinkage as has been seen in any animal.

HISTOLOGICAL EXAMINATION

The pronounced swelling, which occurs in the cat's brain after the intravenous injection of water, and the marked shrinkage which follows the intravenous injection of strongly hypertonic solutions, has led to the desire to correlate, if possible, these gross alterations with histological changes in the cerebral substance.

Mention has already been made in this paper of the fact that fixation with formaldehyde does not preserve accurately the gross form and size relations of the brain as seen prior to death of the animal, so that the preliminary observations here recorded may represent only very roughly the histological picture accompanying the various modifications of brain bulk. That there are marked histological changes is readily seen but their exact interpretation, particularly in regard to the representation of conditions prevailing prior to fixation, is a matter requiring further study. It is hoped that when a method of fixation is devised which will preserve the form and size relations accurately, more intelligent histological and cytological observations may be made. The present findings are reported tentatively, pending an attempt to control the artifacts probably introduced by the technical methods employed.

The material available for this study was that resulting from the experiments described in the preceding parts of this paper. This material was preserved in formaldehyde, which was in most cases injected through the aorta immediately after the death of the animal. One series of brains fixed by Formaline-Zenker's fluid was used in an attempt to control the material preserved in formaldehyde. Sections were cut in paraffin, 10 μ thick; stained with haematoxylin and eosin, toluidine blue and fuchsin S, and with Mallory's and Van Gieson's connective tissue stains. All sections were made from blocks of cortex including the sulcus lateralis and parts of the two adjacent gyri, taken from the dorsal surface of the brain in the same transverse plane as the optic chiasma. In the animals where the skull was opened during the experiment, this block of cortex was found to be in the upper part of the trephine opening. Examination of sections of cortex taken from animals used in this study quickly showed that, when judged by the histological changes which accompany or follow gross shrinkage or swelling, these animals may be divided into two groups; those in which the skull and dura were opened during the experiment and those in which the cerebral cavity remained intact. The histological changes seen in sections of non-decompressed brains following various intravenous injections are quite marked and constant in the material studied, but sections from decompressed brains fail to exhibit the same marked differences from the control. It is quite evident, then, that following decompression the brain may adjust its volume; it may herniate, being only partially restricted by the dura, or collapse freely. This comparative freedom to contract or expand probably explains the finding of similar histological pictures in the normal decompressed controls, and in the decompressed brains following intravenous injection of water or salts. All the specimens tended to approach the normal when the brains were allowed to contract or expand with freedom, but when the alterations in cerebral volume were limited by the closed cranium, the factors responsible for the macroscopic changes in brain bulk produced also marked microscopic changes. Although these histological changes as observed have doubtless been altered by the technical methods employed in the preservation of these brains, their constancy is ample evidence that they are indicative of certain fundamental changes in the brain substance, even though they may represent very inaccurately the actual conditions prevailing prior to death. The technical procedures employed have been practically uniform for all brains, so that the artifacts produced may be considered as constant.

Skull unopened

Normal. Control sections were made from blocks of cortex taken from animals receiving no intravenous injection and from animals in which Ringer's solution was introduced intravenously. While it is apparent that there are artifacts in these sections, due probably to the method of fixation, they furnish a reasonable control for sections of brains taken from animals receiving intravenous injections of various concentrations. An examination of sections made from brains of animals in which Ringer's solution was introduced intravenously and a comparison of these sections with normal controls shows no fundamental differences in the two, so far as our observations go. These sections, then, furnish a reasonable standard with which the sections from animals subjected to other experimental procedures may be compared.

Water. The general appearance of sections of the cortex made from non-decompressed brains following the intravenous injection of water, is that of a swollen tissue. The sulci are quite narrow and the gyri tend to be flat. The smaller vessels seem, if different from normal, contracted within normal or slightly expanded perivascular spaces. The intercellular material of the gray matter seems inflated, the spaces found among the interlacing cell processes appearing larger than in control sections. Many of the large dendrites which rise perpendicular to the free surface of the gyrus are apparently larger than in the normal. Under higher magnifications the nuclei of nerve cells seem compact, the whole nucleus being perhaps slightly contracted. About the cells in most of the water brains examined, there is an evidently enlarged pericellular space, the general impression being that the cell itself is contracted away from the surrounding tissue. The occurrence of enlarged pericellular spaces in the gray matter of the cortex, following the intravenous injection of water, is most striking and constant. Pericellular spaces are apparent in sections of normal cortex, particularly about the larger cells, but following the introduction of water intravenously these spaces, even about the smaller cells, are evidently considerably enlarged. In this material the enlarged pericellular spaces and other histological evidences of change may be interpreted as due to conditions within the brain, different from those existing normally, and produced by the intravenous injection of a hypotonic solution.

Salt. Sections of the cortex taken from brains following intravenous injection of 30 per cent sodium chloride, under low magnification, show in general an appearance of tissue contraction, the sulci being wide and the substance of the brain condensed. In practically all of the salt brains examined, the cortical capillaries seem distended. This distension may be explained partially, perhaps, by the arterial injection with formaldehyde (a procedure carried out with the normal and water brains) but it is a constant finding in the salt brains and may be taken as an indication of some constant factor in the fluid concentration within the nervous tissue. In most of the salt brains the perivascular spaces are apparent but not enlarged. The intercellular felt-work in the gray matter seems compacted and denser than in normal sections. The outstanding feature of the histological picture presented by these sections of salt brains concerns the occurrence of a marked clear space about the nuclei of many cells. These clear spaces vary in size from a slight space about a well-rounded nucleus to a wide space around a markedly crenated nucleus. These spaces increase in size in the gray matter from the medullary core out to the surface layers of each gyrus. The nuclei appear condensed, the chromatin being aggregated. The Nissl substance in the cytoplasm is at the periphery of the cell, the perinuclear spaces being between the nuclear membrane and a marginal ring of Nissl substance. In sections stained with toluidine blue and fuchsin S, these spaces are seen to be partially filled with a fluid-coagulum which stains with fuchsin. This coagulum in most cases has shrunk away from the nucleus toward and against the ring of Nissl substance. No pericellular spaces are usually apparent about the cells showing such perinuclear spaces. The pericellular spaces may be observed occasionally however in the deeper layers of the gray matter about the larger cells. As noted above, the perinuclear spaces are more marked and the nuclei more crenated in cells of the surface layers of the cortex. It seems evident, then, after a comparison of these sections with normal control sections, that in these brains the intracellular perinuclear spaces and other histological evidences of change in the brain substance may be attributed to changes brought about by the intravenous injection of a hypertonic solution.

Skull opened

Examination of sections of brains following intravenous injections of solutions of various concentration in animals with opened skulls reveals a histological picture quite similar in all. There are, of course, minor differences in the sections examined, but such differences do not seem to be due to any changes brought about by the intravenous injection. The decompression has evidently allowed each brain to expand or contract freely and to adjust its fluid distribution so that no essential histological differences are noticeable, all retaining very nearly a normal appearance. That there may be in these brains fundamental histological and cytological differences not revealed by the methods employed, is probable, but further work is necessary to establish such differences. With the methods employed it is certain that the decompressed brains do not show the histological characteristics which are so evident in the non-decompressed brains.

An exact interpretation of the above observations on the histological changes in the cortex of the brain following intravenous injections of hypotonic and hypertonic solutions can not now with reason be attempted. That there are histological changes in brains unrelieved by decompression is certain, but these changes need more accurate study and control before any sane effort can be made to explain them accurately. The changes described have no doubt been influenced by the technical methods employed; these methods may have, in addition, masked or destroyed other histological evidences of changes produced by the intravenous injections. Until one finds a method of fixation which will preserve the form and size relations of the brain accurately and at the same time will make possible accurate histological study, the above observations may be accepted tentatively as an indication that changes in the brain substance, recognizable histologically, do occur following intravenous injections of solutions of various concentrations.

DISCUSSION OF RESULTS

The experimental alteration of the volume of the brain by intravenous injections of hypotonic and hypertonic solutions has not, so far as we have been able to find, been previously recorded in the literature. The ease and rapidity of these changes in brain volume are of considerable interest in view of the old idea of the incompressible character of the brain and its relation to the conception of a constant vascular volume within the cranial cavity.

The hypothesis that the volume of the blood circulating within the cranium must at all times be constant was first brought forward by Alexander Monro, the younger (2) in 1783. At this time he wrote that

> as the substance of the brain, like that of other solids of our body, is nearly incompressible, the quantity of blood within the head must be the same at all times, whether in health or disease, in life or after death, those cases only excepted in which water or other matter is effused or secreted from the blood-vessels; for in these cases, a quantity of blood equal in bulk to the effused matter, will be pressed out of the cranium.

This viewpoint advanced by Monro was accepted and elaborated by Kellie (3) in 1824, who based his ideas upon observations on men frozen to death and upon experiments on animals. His conclusions were that a state of bloodlessness did not exist in the brains of animals killed by bleeding, that the quantity of blood in the cerebral vessels was not affected by posture or gravitation, that congestion of these vessels was not found in those conditions in which it might be well expected (hanging, etc.) and that compensatory readjustments between the different sets of cerebral vessels always maintained a constant vascular volume. Subsequently Kellie wrote

> that in the ordinary state of these parts we can not lessen, to any extent, the quantity of blood within the cranium, by arteriotomy or venesection; whereas if the skull of an animal be trephined then hemorrhage will leave very little blood in the brain.

Within the next two decades following the publication of the results of Kellie's experiments, many clinical observations were reported in substantiation of this conception—that the vascular content of the brain was at all times practically constant. This Monro-Kellie doctrine received wide publicity through its acceptance by Abercrombie (4). This eminent surgeon, in discussing apoplexy, thus summed up his views on the subject (p. 300):

> In this investigation it is unnecessary to introduce the question, whether the brain is compressible, because we may safely assert that it is not compressible by any such force as may be conveyed to it from the heart through the carotid and vertebral arteries. Upon the whole, then, I think we may assume the position as being in the highest degree probable, that, in the ordinary state of the parts, no material change can take place in the absolute quantity of blood circulating in the vessels of the brain.

Burrows in 1843 (5) was probably the first to question the absolute accuracy of this doctrine which so firmly considered the brain as of fixed incompressible bulk. He emphasized strongly the importance of the cerebro-spinal fluid, as the means of replacing the loss of blood during hemorrhage, for he felt that the amount of intracranial blood was obviously diminished by systemic bleeding.

"Whether the vacated space is replaced by serum, or resiliency of the cerebral substance under diminished pressure, is another question" was Burrows' summary of the possible readjustment for variations in the volume of the cerebral blood. As far as can be ascertained, this is the first statement of the view that the volume of the brain may be altered in accord with pathological or physiological conditions within the cranium. Burrows presents one of the most satisfactory conceptions of the whole process of fluid changes within the cranium (p. 32):

> Those who have maintained this doctrine of the constant quantity of blood within the cranium, have not, I believe, taken into due consideration that large proportion of the contents of the cranium which consists of extra-vascular serum. Regarding this serum as an important element of the contents of the cranium, I admit that the whole contents of the cranium, that is, the brain, the blood, and this serum together, must be at all times nearly a constant quantity.

It was only when the subject of a constant blood volume in the cranium was subjected to experimental test that reliable data were obtained. Kussmaul and Tenner (6) demonstrated the unreliability of post-mortem observations and came to the conclusion, advanced by Burrows and supported by the experimental work of Donders (7), that variations in the total volume of blood in the cranium occurred. These early experiments, as pointed out by Leonard Hill (8) in 1896, are not conclusive as variation in the blood volume in one part of the cerebral vascular system might well be compensated by readjustments in another. Following many other investigators who used various methods of experimental attack, Leonard Hill concluded that (p. 77): "The volume of blood in the brain is in all physiological conditions but slightly variable."

More recently (1914) Dixon and Halliburton (9), in the course of an extensive study of the cerebro-spinal fluid, have come to the conclusion "that the cranial contents cannot any longer be regarded as a fixed quantity without the power of expanding or contracting in volume."

It must be assumed, however, that with certain reservations, the data favor the idea of a relatively fixed total volume of the cranial contents but with the capacity for change in any one of the three chief elements concerned.

The conception of a more or less constant cranial content is closely related to the questions which are naturally called forth by the experimental modification of brain bulk, detailed in foregoing sections of this paper. For within the closed cavity the alteration in volume of any element must be at the expense of the other elements. First of the possible explanations of the experimental alteration in brain bulk is that relating to the blood volume of the cranium. Are the vascular readjustments following the intravenous injection of hypotonic and hypertonic solutions sufficient to account for the definite change in brain bulk? In one of his original experiments, in which the cranium of a dog had been trephined, Kellie observed a recession of the brain away from the skull during exsanguination. Ecker (9) also observed in a trephined animal a remarkable shrinkage of the brain when the loss of blood from division of the carotids became excessive. The converse of this vascular diminution of the brain volume was recorded also by Ecker, who found that pressure on the thorax of a trephined dog caused protrusion of the brain into the cranial opening. Burrows also comments on this possibility of hernia through the trephine opening occurring in those cases in which the blood supply to the brain was markedly increased. These early observations on the relation of the cranial vascular supply to the volume of the brain during life have been confirmed and substantiated by many other workers on the cerebral circulation and cerebro-spinal (intracranial) pressure.

In our own experiments the modification of brain bulk has been produced both in the opened and in the intact skull. Observations on venous and arterial pressures under such experimental conditions have been made; these will be reported at another time. But that these alterations in brain bulk are independent of changes in volume of the blood in the vascular bed, may be deduced from other findings. The fact that similar changes occur in both the trephined and the unopened skull is strong evidence against the view that these changes in brain bulk depend on alterations in vascular volume and the persistence of the anatomical change after formalin fixation makes such a view untenable. For it must be assumed that with death of the animal, opening of the chest, introduction of a cannula in the aorta, injection of formalin through this vessel and release of the pressure by incising the right auricle, any vascular alterations existing in life are no longer maintained; so that the persistence, after such fixation, of a given brain bulk, if due simply to the amount of blood in the capillary bed and other vessels of the brain, would be impossible. That changes in bulk do persist after fixation as above, is ample evidence that such changes are maintained by some fundamental and comparatively stable alterations in the substance of the brain itself. A further fact, which shows that the changes in volume of the brain as produced in these experiments are fundamentally independent of vascular alterations, is that brains fixed by immersion in formalin, as well as those preserved by arterial injection of the fixing agent, retain in part the changes in bulk produced by intravenous injection. Following several experiments in which, after opening of the skull the brain bulk was changed by intravenous injection, the heads of the animals were cut from the body and immersed in formalin. That the skull and dura were freely open during experimentation in these cases and that all the vessels of the neck were severed before fixation by immersion and not by injection and that after such treatment the brain still maintained the volume change brought about by the intravenous injection, are further evidence that the changes in bulk are independent of vascular alterations. While, as has been emphasized, the method of preservation does not accurately maintain the condition existing in life, it nevertheless makes possible the recognition of undeniable evidence of change in brain bulk. Vascular alterations may account for some changes in brain bulk which occur in the living animal, but the changes persisting in death and after the technical procedures employed in this investigation are quite evidently due to some other cause.

The other variable factor which may operate within the cranium in producing changes in brain bulk involves the cerebro-spinal fluid. We have already noted the lasting rise in the pressure of the cerebro-spinal fluid following the intravenous injection of a hypotonic solution, and the production of swollen brains by such injections. Conversely, a definite decrease in the size of the brain has been found in those cases in which the pressure of the cerebro-spinal fluid has been markedly lowered by intravenous injection of hypertonic solutions. In considering these results it becomes rather difficult to determine with absolute accuracy the primary factor involved in producing these alterations. Does the modification of the bulk of the brain determine the pressure-change in the cerebro-spinal fluid or are both dependent individually on some more fundamental cause? That change in brain volume in our experiments is not caused alone by changes in the pressure of the cerebro-spinal fluid is demonstrated by its occurrence in the opened skull, for with the trephine opening and the dura incised, the fluid pressure becomes minimal, and any rise in pressure is within certain limits relieved. That fundamental osmotic changes in the blood are responsible for the changes in the pressure of the cerebro-spinal fluid, following intravenous injections of solutions of various tonicity, seems a reasonable conclusion. Although it is probable that change in the volume of the brain may affect the pressure of the cerebro-spinal fluid, and possible that changes in the pressure of the fluid may alter the bulk of the brain, in these experiments there is evidence that primary alterations in brain bulk and cerebro-spinal fluid pressure, both, are caused by fundamental osmotic changes in the blood supplied to the brain.

Such considerations force one to conclude that the alteration in the volume of the brain following intravenous injections of hypotonic or hypertonic solutions is quite independent of change in either the volume of the blood supply to the brain or of the pressure of the cerebro-spinal fluid. With the diminution in bulk the pressure of the cerebro-spinal fluid falls—a partial compensation for the evacuated space formerly occupied by the brain of normal size. Conversely, an increased bulk of the brain may cause dislocation of a certain volume of the cerebro-spinal fluid, thus raising its pressure as determined in a manometer.

Relating this experimental modification of the brain bulk to the restricted Monro-Kellie doctrine it becomes evident that another variable factor must be introduced. The brain should no longer be considered as incompressible and of fixed volume as the early writers assumed it to be, but as subject to variation in size under experimental conditions. Monro, of course, qualified his theory by consideration of matter "effused or secreted from the blood vessels," and Burrows suggested that the brain possessed "resiliency of the cerebral substance under diminished pressure." Pathologically the increase in bulk of the brain is well known in the cerebral edemas of trauma, acute infections and certain other conditions. Similarly, pathological states characterized by diminished volume of the brain are also quite common. The Monro-Kellie doctrine then requires marked modification; the view so well advanced by Burrows is probably the more correct. This leads one to assume that the cranial cavity is relatively fixed in volume and is completely filled by brain, cerebro-spinal fluid and blood; variations in any one of the three elements may occur, compensation being afforded by alteration in the volume of one or both of the remaining elements.

The underlying processes involved in the modification of brain bulk by the intravenous injection of hypertonic and hypotonic solutions seem concerned then with osmotic changes in the blood. That the osmotic pressure of the blood is an essential factor in such experimental changes in brain bulk is shown by the fact that no alteration in the volume of the brain follows relatively large doses of Ringer's solution (100 cc. in a cat) but occurs promptly on intravenous injection of far smaller amounts of distilled water or concentrated salines. Just how this change in osmotic value of the blood affects the brain tissue and alters its volume can only be speculated upon at the present time. The change is limited apparently by the potential distensibility or contractility of the brain in the particular animal used. Thus, in old cats, the alterations in brain volume have not been so marked as in younger animals, though the contractility seems to persist longer than the distensibility. On the other hand, in young animals the change in cerebral volume is of far easier accomplishment. Capacity for osmotic changes in these animals must be about the same; the resultant modification of the brain bulk then is limited by anatomical factors. Finally, in the closed skull certain changes take place, limited by the potential powers of change in the brain itself and by the intradural capacity; in the trephined skull, the only limitation to change is the intrinsic capability for contraction or expansion of the brain itself.

SUMMARY

1. Intravenous injection of a hypertonic solution (30 per cent NaCl or saturated $NaHCO_3$) is followed by a marked decrease in size of the brain; when the skull is opened the brain may be seen to fall away several millimeters from the inner surface of the skull after such injection.
2. Intravenous injection of a hypotonic solution (water) causes a marked swelling of the brain; when openings are made in the skull the brain will rise, forming tense herniae protruding several millimeters through the trephine openings.
3. These changes are independent of the volume of the fluid injected and are probably due to fundamental osmotic effects of the hypotonic and hypertonic solutions.
4. The brains of old cats fail to respond readily to intravenous injection, particularly to the intravenous injection of hypotonic solutions.
5. Internal changes, recognizable histologically, have been found quite constantly in the brains of animals which have been given intravenous injections of hypertonic or hypotonic solutions and which have not been trephined. On the contrary, in animals in which the skull is opened and the brain thus allowed to change its volume freely, these histological changes have not been demonstrated.

BIBLIOGRAPHY

(1) **Weed and McKibben**: This Journal, 1919, xlviii, 512.
(2) **Monro**: Observations on the structure and function of the nervous system, Edinburgh, 1783.
(3) **Kellie**: On death from cold and congestion of the brain, Edinburgh, 1824. Reprint from Trans. Med. Chir. Soc.
(4) **Abercrombie**: Pathological and practical researches on diseases of the brain, etc., Edinburgh, 1828.
(5) **Burrows**: On disorders of the cerebral circulation, etc., (London, 1846); Philadelphia, 1848.
(6) **Kussmaul and Tenner**: On the nature and origin of epileptiform convulsions, etc. Trans. by Bronner, New Sydenham Society, London, 1859.
(7) **Donders**: Schmidt's Jahrbuch, 1851, lxix, 16.
(8) **Hill**: The physiology and pathology of the cerebral circulation, London, 1896.
(9) **Dixon and Halliburton**: Journ. Physiol., 1914, xlviii, 128.
(10) **Ecker**: Physiol. Untersuch. über die Bewegungen des Gehirns, etc., Stuttgart, 1843.

PLATE I

Fig. 1. Photograph × ¾. Cat no. 1304. Adult female. Occipito-atlantoid puncture. Continuous observation of pressure of cerebro-spinal fluid for 75 minutes, with intravenous injection of 35 cc. sterile distilled water. Pressure rose from 105 mm. to 175 mm. following injection. Sacrificed with ether 30 minutes after completion of injection. Fixed by injection with 10 per cent formalin. Dura not removed.

Fig. 2. Photograph × ¾. Cat no. 1371. Adult male. Intravenous injection of 20 cc. 30 per cent NaCl (Squibb) in 5 cc. doses given at 30 minute intervals. Sacrificed with ether 2 hours after first and 30 minutes after last dose. Fixed by injection with 10 per cent formalin. Dura not removed.

Fig. 3. Photograph × 1. Cat no. 1402. Adult female. Control. Well-nourished, normal animal. Sacrificed with ether. Transverse section through optic chiasma. Fixed by injection with 10 per cent formalin.

Fig. 4. Photograph × 1. Cat no. 1309. Young adult female. Occipito-atlantoid puncture. Continuous observation of pressure of cerebro-spinal fluid for 81 minutes, with intravenous injection of 12 cc. Ringer's solution. Initial pressure 90 mm., final pressure 110 mm. Sacrificed with ether 1 hour after completion of injection. Transverse section through optic chiasma. Fixed by injection with 10 per cent formalin.

Fig. 5. Photograph × 1. Cat no. 1304. Transverse section through optic chiasma of brain shown in figure 1.

Fig. 6. Photograph × 1. Cat no. 1371. Transverse section through optic chiasma of brain shown in figure 2.

Fig. 7. Photograph × 1. Cat no. 1303. Adult female. Occipito-atlantoid puncture. Continuous observation of pressure of cerebro-spinal fluid for 84 minutes, with intravenous injection of 20 cc. sterile distilled water. Pressure rose from 106 mm. to 195 mm. Sacrificed with ether 1 hour after completion of injection. Transverse section through optic chiasma. Fixed by injection with 10 per cent formalin.

Fig. 8. Photograph × 1. Cat no. 1364. Young adult female. Occipito-atlantoid puncture. Continuous observation of pressure of cerebro-spinal fluid for 90 minutes, with intravenous injection of 10 cc. saturated aqueous solution of sodium bicarbonate. Pressure fell from 115 mm. to below zero. Sacrificed with ether 70 minutes after completion of injection. Transverse section through optic chiasma. Fixed by injection with 10 per cent formaldehyde.

PLATE II

Fig. 9. Photograph × ¾. Cat no. 1524. Adult female. Weight 2,350 grams. Temporal decompression on left side. Intravenous injection of 100 cc. sterile distilled water. Marked hernia of brain through trephine opening 3 mm. beyond outer table of skull beginning with the injection and persisting until animal was sacrificed with ether 35 minutes after completion of injection. Fixed by immersion with 10 per cent formalin. Trephine opening made on right side after the beginning of fixation.

Fig. 10. Photograph × ¾. Cat no. 1531. Adult male. Weight, 2,330 grams. Temporal decompression on left side. Intravenous injection of 100 cc. Ringer's solution. Brain lies convex with no hernia or evidence of swelling or shrinkage throughout the experiment. Sacrificed with ether 36 minutes after completion of injection. Fixed by immersion with 10 per cent formalin. Trephine opening made on right side 45 minutes after the beginning of fixation.

Fig. 11. Photograph × ¾. Cat no. 1506. Adult male. Weight, 2,100 grams. Temporal decompression on left side. Intravenous injection of 16⅓ cc. 30 per cent NaCl (Squibb). Brain rises slightly with beginning of injection but falls away from skull before injection is finished. Thirty minutes later lies concave, 3 mm. below inner table of skull at forward edge of opening. Sacrificed with ether 33 minutes after completion of injection. Fixed by immersion with 10 per cent formalin. Trephine opening made on right side after the beginning of fixation.

Fig. 12. Photograph × 1. Cat no. 1501. Adult female. Weight 2,400 grams. Temporal decompression on left side. Intravenous injection of 100 cc. sterile distilled water. Brain rises as injection begun; hernia increases during injection; brain bulges markedly through trephine opening with protrusion in center about 8 mm. beyond outer table of skull, 35 minutes after injection finished. Sacrificed with ether 37 minutes after completion of injection. Fixed by injection through aorta with 10 per cent formalin. Transverse section just behind optic chiasma. Dura removed.

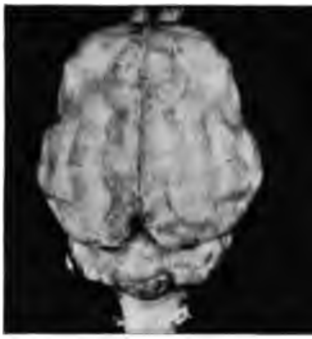
Fig. 13. Photograph × 1. Cat no. 1503. Adult female. Weight 2,260 grams. Control. Temporal decompression on left side. No intravenous injection. Brain lies convex about level with the outer table of the skull throughout experiment. Sacrificed with ether 35 minutes after completion of decompression. Fixed by injection through aorta with 10 per cent formalin. Transverse section just behind optic chiasma. Dura removed.

Fig. 14. Photograph × 1. Cat no. 1505. Adult female. Weight 2,050 grams. Temporal decompression on left side. Intravenous injection of 16⅓ cc. 30 per cent NaCl (Squibb). Brain bulges markedly during injection, but immediately begins to subside on completion of injection until in 30 minutes it lies concave 1 mm. below inner table of skull. Sacrificed with ether 36 minutes after completion of injection. Fixed by injection through aorta with 10 per cent formalin. Transverse section just behind optic chiasma. Dura removed.

Fig. 15. Photograph × 1. Cat no. 1536. Adult female. Weight 2,430 grams. Double temporal decompression. Intravenous injection of 100 cc. sterile distilled water. Beginning with the injection brain rises in a tense hernia on both sides. Sacrificed with ether 31 minutes after completion of injection. Fixed by injection through aorta with 10 per cent formalin. Transverse section through optic chiasma. Dura removed.

Fig. 16. Photograph × 1. Cat no. 1532. Adult male. Weight 2,250 grams. Control. Double temporal decompression. No intravenous injection. Throughout experiment brain lies convex with no bulging. Sacrificed with ether 20 minutes after completion of decompression. Fixed by injection through aorta with 10 per cent formalin. Transverse section through optic chiasma. Dura removed.

Fig. 17. Photograph × 1. Cat no. 1541. Young male. Weight 1,500 grams. Double temporal decompression. Intravenous injection of 16 cc. 30 per cent NaCl (Squibb). Beginning with injection brain falls away from skull lying about 3 mm. below inner table 20 minutes after injection stopped. Sacrificed with ether 55 minutes after completion of injection. Fixed by injection through aorta with 10 per cent formalin. Transverse section through optic chiasma. Dura removed.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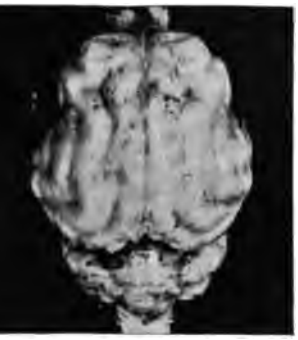
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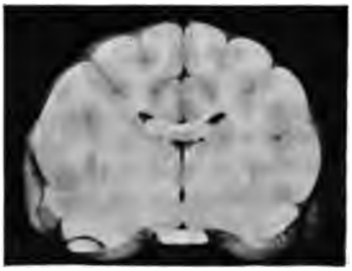
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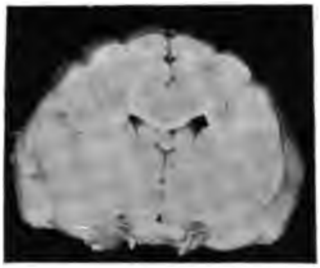
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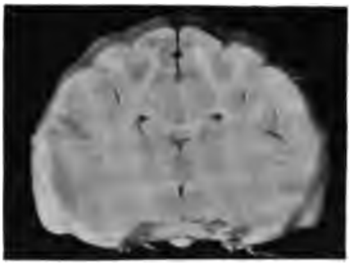
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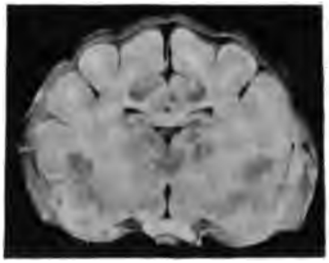
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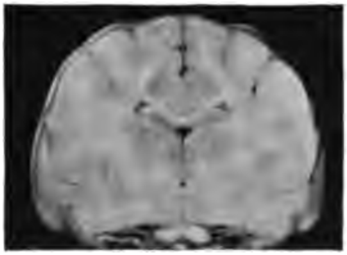
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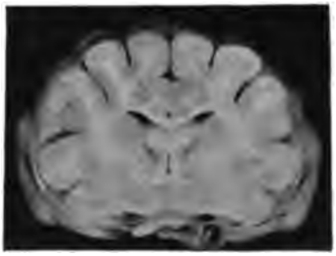
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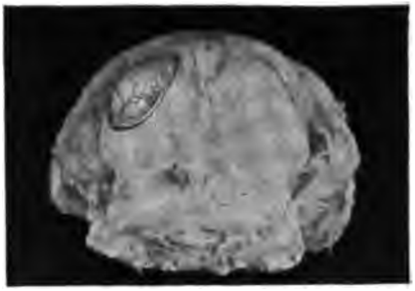

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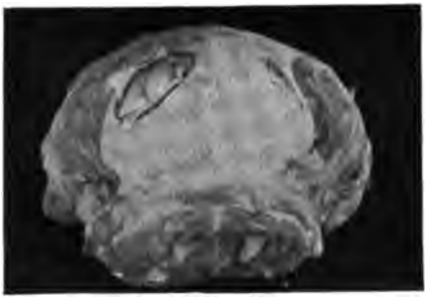

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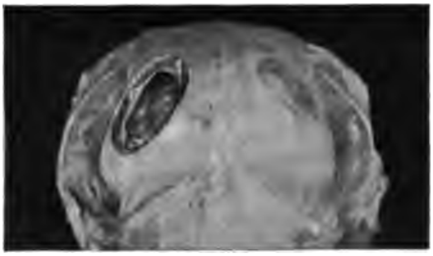

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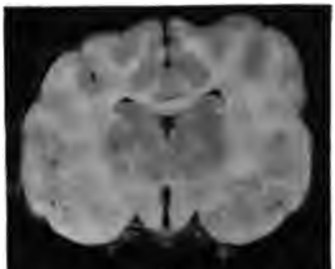

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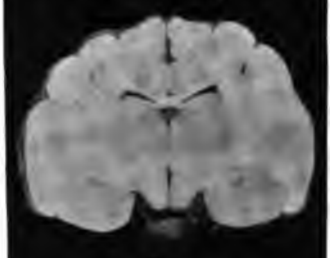

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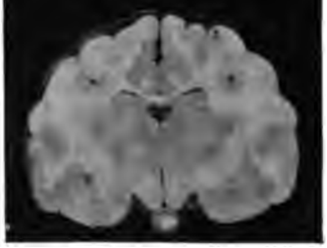

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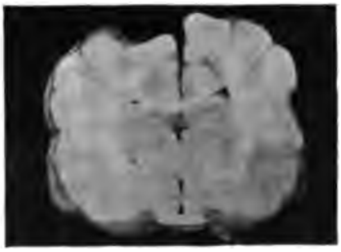

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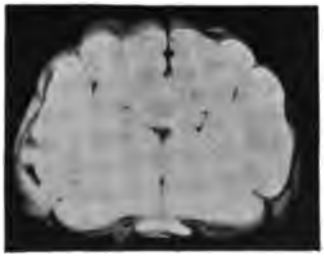

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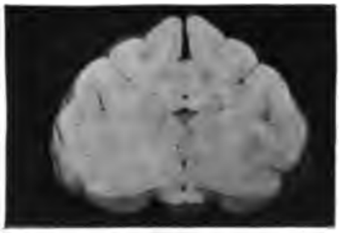

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